UNITED STATES DISTRICT COURT
NORTHERN DISTRICT OF ILLINOIS
EASTERN DIVISION

Ann Coyle, on behalf of herself and all others similarly situated, Plaintiff, v. Avent America, Inc.; Philips Electronics North America Corporation; Gerber Products Company; Handi-Craft Company; Nalge Nunc International Corp.; Playtex Products, Inc.; Defendants.	No. **COMPLAINT – CLASS ACTION** **JURY DEMAND** FILED: JUNE 12, 2008 08CV3407 JUDGE HOLDERMAN MAGISTRATE JUDGE COX TC

1. Plaintiff, by her attorneys, brings this Class Action against Defendants Avent America, Inc., Gerber Products Company, Handi-Craft Company, Nalge Nunc International Corp., and Playtex Products, Inc. on her own behalf and on behalf of a class of all other similarly situated purchasers within Illinois and the United States, including all persons who purchased and/or acquired bottles or cups manufactured, sold and/or distributed by Defendants from May 2003 through the present ("Class Period"), which contained bisphenol A ("BPA"). BPA is a chemical produced in large quantities for use in the production of polycarbonate plastics and epoxy resin. BPA is found in certain food and drink packaging or containers, such as infant bottles and cups. BPA is also a developmental, neural and reproductive toxicant that is typically

described as being "estrogenic," which means that it acts like the hormone estrogen. Scientists have linked even very low doses of BPA exposure to cancers, impaired immune function, early onset of puberty, obesity, diabetes, and hyperactivity, among other problems. Plaintiff brings this action for compensatory damages and for equitable, injunctive, and declaratory relief against Defendants, who designed, manufactured, marketed, sold and/or distributed products intended for young children containing BPA ("Hazardous Baby Products"). Plaintiff alleges the following upon her own knowledge, or where there is no personal knowledge, upon information and belief and the investigation of her counsel:

I. NATURE OF THE ACTION

2. For years Defendants have manufactured and sold plastic baby bottles and/or training or "sippy" cups containing BPA, despite the scientific consensus that BPA poses an unacceptable risk to infants and young children. Despite Defendants' awareness of the risks of using BPA in products intended for use as food or drink containers for young children, Defendants continue to manufacture and sell Hazardous Baby Products to parents and to represent these products as safe. Even though safe alternatives to BPA are available, Defendants have chosen to prioritize their bottom lines over children's health and continue to manufacture and sell BPA-laced products.

3. As a parent who is ceaselessly exposed to a media advertising blitz touting the safety and effectiveness of Defendants' products, Plaintiff trusted that Defendants' brand-name feeding products were safe for children. Plaintiff placed her trust in companies, like Defendants, which hold themselves out as experts on caring for the health of infants and young children.

4. Young children are especially at risk because for any given exposure, a smaller body size receives a greater effect and a larger dose. In addition, young children's developing body systems are extremely susceptible to chemical disruptions.

5. Defendants' Hazardous Baby Products are unsuitable for use. The health impacts of these products are serious enough to cause governments around the world, such as Canada, to begin the process of banning such products. Yet, as of the date of this filing, Defendants' Hazardous Baby Products are still on the shelves, and Defendants still tout their safety and healthfulness.

6. Under appropriate legal standards, and especially as viewed in the eyes of a "reasonable parent," Defendants' marketing is deceptive; their products are unsuitable for use; and their actions are unconscionable. Defendants manufactured and/or sold Hazardous Baby Products and should be required to compensate Plaintiff and similarly situated consumers for their damages.

II. PARTIES

A. PLAINTIFF

7. Plaintiff Ann Coyle is a resident of Highland Park, Illinois. Plaintiff purchased baby bottles manufactured by Defendants Avent America, Inc. and Handi-Craft Company (marketed as "Dr. Brown's" bottles), in approximately January, 2005. Plaintiff purchased these products for her young child, then age four months. Plaintiff purchased training cups from Defendants Avent America, Inc., Nalge Nunc International Corp., and Playtex Products, Inc. in approximately December, 2005. Plaintiff purchased these products for her young child, then age fifteen months. When Plaintiff purchased the bottles and cups for her children, she did not intend to expose them to BPA through the use of these products.

8. Defendant Avent America, Inc. ("Avent") is a corporation organized and existing under the laws of the state of Illinois which maintains its principal place of business in Bensenville, Illinois. Avent manufactures plastic baby bottles, nipples, training cups, and/or other products that contain BPA. Avent has conducted and continues to conduct business in Illinois by distributing for sale and selling its products through various stores or supermarkets located in Illinois.

9. Defendant Philips Electronics North America Corporation is a corporation organized and existing under the laws of the state of Delaware which maintains its principal place of business in New York, New York. In September 2006, Philips Electronics North America Corporation acquired Avent, and now manufactures plastic baby bottles, nipples, spill-resistant cups, and/or other products that contain BPA. These products are marketed under the Philips AVENT brand. Philips Electronics North America Corporation has conducted and continues to conduct business in the State of Illinois by distributing for sale and selling its products through various stores located in Illinois.

10. Defendant Gerber Products Company ("Gerber") is a corporation organized and existing under the laws of the state of Michigan which maintains its principal place of business in Parsippany, New Jersey. Gerber manufactures plastic baby bottles, nipples, training cups, and other products that contain BPA. Gerber has conducted and continues to conduct business in Illinois by distributing for sale and selling its products through various stores and supermarkets located in Illinois.

11. Defendant Handi-Craft Company ("Handi-Craft") is a corporation organized and existing under the laws of the state of Missouri which maintains its principal place of business in St. Louis, Missouri. Handi-Craft manufactures plastic baby bottles and other products that

contain BPA under the brand name Dr. Brown's. Handi-Craft represents that Dr. Brown's bottles have a patented venting system, creating "positive pressure flow, just like breastfeeding." Handi-Craft has conducted and continues to conduct business in Illinois by distributing for sale and selling its products through various stores, supermarkets, and on-line retailers located in Illinois.

12. Defendant Playtex Products, Inc. ("Playtex") is a corporation organized and existing under the laws of the state of Delaware which maintains its principal place of business in Westport, Connecticut. Playtex manufactures plastic baby bottles, nipples, training cups, and/or other products that contain BPA. Playtex has conducted and continues to conduct business in Illinois by distributing for sale and selling its products through various stores and supermarkets located in Illinois.

13. Defendant Nalge Nunc International Corporation ("NNIC") is a corporation organized and existing under the laws of Delaware which maintains its principal place of business in Rochester, New York. Through its Outdoor Products Division, located in Rochester, New York, NNIC manufactures, markets and sells a variety of reusable plastic beverage containers, including the Grip'n Gulp™, a popular training cup for toddlers. NNIC has conducted and continues to conduct business in Illinois by distributing for sale and selling its products through various outdoor stores, and on-line retailers located in Illinois.

III. JURISDICTION AND VENUE

14. This Court has jurisdiction over the subject matter of this action pursuant to 28 U.S.C. § 1332, as amended by the Class Action Fairness Act of 2005, because the matter in controversy exceeds $5,000,000, exclusive of interest and costs, and is a class action in which some members of the Class are citizens of different states than the Defendants. *See* 28 U.S.C.

§ 1332(d)(2)(A). This Court has supplemental jurisdiction over the state law claims pursuant to 28 U.S.C. § 1367. This Court has personal jurisdiction over Defendants because they are authorized to do business and to conduct business in Illinois. Defendants have specifically marketed and sold the Hazardous Baby Products in Illinois, and they have sufficient minimum contacts with this state and/or sufficiently avail themselves to the markets of this state through their promotion, sales, and marketing within this state to render the exercise of jurisdiction by this Court permissible.

15. Furthermore, Plaintiff alleges that more than two-thirds of all of the members of the proposed Class in the aggregate are citizens of states other than Illinois, and that the total number of members of the proposed Class is greater than 100, pursuant to 28 U.S.C. § 1332(d)(5)(B).

16. Venue in this Court is proper pursuant to 28 U.S.C. § 1391(a) because Plaintiff resides in this District, a substantial part of the events giving rise to the claims asserted herein occurred in this District, and Defendants are subject to personal jurisdiction to the federal court in this District. Moreover, Defendant Avent is an Illinois corporation and other Defendants inhabit and/or may be found in this judicial district and the interstate trade and commerce described herein is and has been carried out in part within this judicial district.

IV. DEFENDANTS' BOTTLES, CUPS, AND CONTAINERS ARE HAZARDOUS TO CHILDREN'S HEALTH

A. THE DANGERS OF BPA EXPOSURE TO YOUNG CHILDREN

17. BPA is most commonly used to make clear polycarbonate plastic for consumer products, including baby bottles and cups. Through use, and especially through dishwasher cleaning and heating baby formula, milk and other beverages and foods, the plastic in baby bottles and other containers breaks down and leaches BPA into liquids and food.

18. Defendants have made calculated and profit-driven decisions to continue manufacturing and marketing baby products containing the industrial chemical BPA, despite their knowledge that their target consumers, i.e., new parents and the caregivers of young children, would not purchase and/or use these products if they were notified of this ingredient and the serious harm it can cause their children. Even as the scientific consensus emerged in the late 1990s regarding the inappropriateness of using BPA in baby bottles, training cups, pacifiers, and other baby products, Defendants continued to manufacture and market these products without notice to new parents. Defendants sold these products throughout the State of Illinois and the United States.

19. New parents have every reason to be concerned about the safety of Hazardous Baby Products and to discontinue the use of such products that they have purchased. Recent publicity and government action have put new parents and other caregivers on notice of the scientific-consensus that Hazardous Baby Products pose an unacceptable risk to children.

20. BPA is an endocrine disruptor; it is an estrogen receptor agonist (a substance that binds to a specific receptor and triggers a response in the cell), and such agonists can act like the body's own hormones, leading to similar physiological effects on the body. Exposure to environmentally relevant doses of BPA have been linked by laboratory studies to a variety of reproductive effects in animals, including reduced sperm production, alterations in prostate development in males, and increased rates of prostate cancer and alterations in mammary gland organization, brain development, and estrous cyclicity in females.

21. A new draft report published by the National Toxicology Program, a division of the U.S. Department of Health and Human Services, states: "[T]he possibility that bisphenol A may alter human development cannot be dismissed." According to the draft report, it is possible

that exposure to BPA during infancy could cause changes in prostate and mammary tissue that raise the risk of cancer later in life. *See* Draft NTP [National Toxicology Program] Brief on Bisphenol A [CAS No. 80-05-7], April 14, 2008 (Exhibit A).

22. Contamination or leaching of BPA into food and beverages occurs because the bond linking BPA monomers to one another to form polymer chains is unstable. This causes the polymer to decay over time. When liquid, such as baby formula or breast milk, contacts plastic bottles or spill-resistant cups containing BPA, the chemical is released into the liquid and the baby drinks it. High heat, such as the heat used when bottles are sterilized *according to Defendant manufacturers' instructions*, accelerates the leaching of BPA from plastic into baby formula or other liquid. Nonetheless, Defendants continue to recommend cleaning their products by boiling, or by putting them in a steam sterilizer (which Defendants Avent and Handi-Craft also sell) or the dishwasher.

23. Since at least 1997, scientists have been concerned about the harmful effects of BPA. A substantial number of scientific studies and reports have shown BPA to be potentially toxic, even at very low doses. Recent studies have confirmed the significant health risks associated with very low levels of BPA exposure. *See* R. Steinmetz et al., *The Xenoestrogen Bisphenol A Induces Growth, Differentiation, and C-Fos Gene Expression in the Female Reproductive Tract*, Endocrinology. 1998 Jun;139(6):2741-7; S. Honma et al., *Low Dose Effect of In Utero Exposure to Bisphenol A and Diethylstilbestrol on Female Mouse Reproduction,"* Reproductive Toxicology, 16:117- 122, 2002; K. Kubo et al., *Low Dose Effects of Bisphenol A on Sexual Differentiation of the Brain and Behavior in Rats*, Neuroscience Research, 45(3):345-56, March 2003.

24. Consumer Reports performed a revealing test on baby bottles back in 1999. The magazine purchased six plastic baby bottles and heated plastic from each in simulated baby formula. It found that the plastic from each of these bottles leached BPA. Consumer Reports calculated that an infant who drank formula from a bottle sterilized by heat would be exposed to a BPA dose of about four percent of an amount shown to affect animals in a laboratory. While this may seem to be a low exposure risk, safety limits for infant exposure are often set as low as 0.1 percent of the level demonstrated to harm laboratory animals. By this standard, Consumer Reports concluded that infants who used the type of bottles tested could be exposed to a BPA dose 40 times higher than the level frequently derived using conservative methods to determine safe levels. Consumer Reports concluded on the basis of its tests and the heightened concern expressed by scientists over the sensitivity of infants to the estrogen-like effects of chemicals such as BPA that the U.S. Food and Drug Administration should re-evaluate the safety of BPA. *See* Consumers Union, *Baby Alert: New Findings About Plastics,* Consumer Reports, 64:28, May, 1999.

25. In 2003, Norwegian researchers conducted a study and detected BPA leaching in 12 polycarbonate baby bottles subjected to simulated use (dishwashing, brushing and scrubbing, and boiling). The level of BPA found in liquids in these bottles exceeded 8 micrograms per liter. *See* C. Brede et al, *Increased Migration Levels of Bisphenol A from Polycarbonate Baby Bottles After Dishwashing, Boiling and Brushing*, Food Additives and Contaminants 20(7): 684-9, 2003.

26. After the Norwegian study, the Environment California Research and Policy Center selected five of the most popular baby bottles, including bottles manufactured by Defendants Avent, Gerber, Handi-Craft and Playtex, and performed its own study ("California Study") to determine whether BPA is released into liquids in these bottles. This study confirmed

the findings of the Norwegian study and reported that "all five bottles leached BPA at varying levels in the same range detected in the Norway study." BPA leaching was found at levels determined to have harmful effects on laboratory animals in numerous studies. The California Study detected 7.74 – 10.07 ppb (parts per billion) of BPA in the Avent (Natural Feeding) baby bottle, 6.07 – 7.07 ppb of BPA in the Dr. Brown's (Natural Flow) baby bottle, 4.58-5.79 ppb of BPA in the Gerber (Premium Feeding System) baby bottle and 4.58 – 5.79 ppb in the Playtex (VentAire) baby bottle (Exhibit B).

27. As indicated in the California Study, children are particularly susceptible to the harmful effects of endocrine disrupters like BPA. However, many of the problems associated with these chemicals, including BPA, cannot be recognized until years after exposure. Health problems caused by BPA can occur over a child's lifespan; thus, it is difficult to determine whether or when health issues related to childhood exposure to BPA will manifest. Limitations on childhood exposure to products containing BPA are critical to prevent or minimize harm to children's intellectual abilities and growth, as well as potential for exposure-related disease. *See* Exhibit B.

28. The United States Centers for Disease Control performed a study in 2003-2004 that confirmed the results of the California Study and observed BPA levels between 0.1 and 9 ppb, which equal or exceed concentrations known to cause adverse effects in laboratory experiments. *See* A. Calafat et al., *Urinary Concentrations of Bisphenol A and 4-Nonylphenol in a Human Reference Population*, Environmental Health Perspectives 113(4): 391-395, April 2005.

29. A paper published in 2007 described a study that evaluated whether BPA migrated into water stored in new or used high-quality polycarbonate bottles. BPA was found to

migrate from polycarbonate water bottles at rates ranging from 0.20 ng/h to 0.79 ng/h. Exposure to boiling water increased the rate of BPA migration by up to 55-fold. *See* H. Le et al., *Bisphenol A is Released from Polycarbonate Drinking Bottles and Mimics the Neurotoxic Actions of Estrogen in Developing Cerebellar Neurons*, Toxicology Letters 176(2):149-56, Jan. 30, 2008.

30. Some researchers have concluded that BPA behaves like a female sex hormone, similar to estrogen. This research, including numerous tests on lab mice, has shown that embryonic and infant mice exposed to small amounts of BPA tend to be obese as adults and that BPA exposure could be a cause of the current rise of human obesity. BPA can also cause increased prostate size, decreased sperm production and increased aggression in male mice. Leaching of BPA into food and beverages held in polycarbonate plastic containers has led to widespread human exposure posing a threat to human health. In his commentary published in 2005, Professor Frederick vom Saal documented a large number of recently published studies showing that the exposure of experimental animals to "low" doses of BPA, still resulting in tissue levels within and even below the range of current human exposure, has been related to adverse effects. *See* Frederick S. vom Saal and Claude Hughes, *An Extensive New Literature Concerning Low-dose Effects of Bisphenol-A Shows the Need for a New Risk Assessment.* ENVIRONMENTAL HEALTH PERSPECTIVES, August, 2005, at 926 (Exhibit C).

31. As of December 2004, 94 of 115 published in vivo studies concerning low dose effects of BPA reported significant effects. Professor Frederick vom Saal and his colleagues also expressed the belief that a new risk assessment for BPA is overdue based on conclusions that the extensive new literature reports adverse effects in animals at doses below the current reference dose; that the high rate of leaching of BPA from food and beverage containers has lead to

widespread human exposure; that the median BPA level in human blood and tissues, including in human fetal blood, is higher than the level that causes adverse effects in mice; and that recent epidemiologic evidence indicates that BPA is related to disease in women. *See* Exhibit C.

32. As summarized by Professor Frederick vom Saal and his colleagues, studies have associated BPA with changes in the brain, pancreas, thyroid function, hormone levels and behavior, prostate and breast cancer, lowered sperm count, early puberty, as well as increased insulin secretion, which can lead to diabetes, obesity, and hypertension. Despite growing and extensive scientific literature reporting adverse health effects from BPA exposure at very low doses, the U.S. chemical industry, including Defendants, continue to resist the idea that BPA is dangerous. *See* Exhibit C.

33. In 2003, researchers discovered that BPA can cause chromosomes to sort incorrectly, even at very low doses. This effect can cause serious genetic health problems, including birth defects such as Down Syndrome and miscarriages. Patricia A. Hunt, et al, *Bisphenol-A Exposures Causes Meiotic Aneuploidy in the Female Mouse*, CURRENT BIOLOGY, April 1, 2003, at 546 (Exhibit D). *See also,* Daniel J. DeNoon, *Danger in Plastic Baby Bottles? Common Plastics Chemical Linked to Genetic Damage,* WebMD Medical News, March 31, 2003, *available at* http://www.webmd.com/baby/news/20030331/danger-in-plastic-baby-bottles (last visited June 4, 2008).

34. Other studies indicating BPA's threat to human health include the following:

- C. Gupta, *Reproductive Malformation of the Male Offspring Following Maternal Exposure to Estrogenic Chemicals*, Proceedings of the Society for Experimental Biology and Medicine 224:61-68 (2000) (BPA linked to

low sperm counts, hyperactivity, early puberty, obesity, small testes size, and enlarged prostates);

- B.S. Rubin et al, *Perinatal Exposure to Low Doses of Bisphenol A Affects Body Weight, Patterns of Estrous Cyclicity, and Plasma LH Levels*, Environmental Health Perspectives 109:675-680 (2001) (BPA exposure makes rodents grow larger and effects persist long after exposure);
- H. Masuno et al, *Bisphenol A in Combination with Insulin Can Accelerate the Conversion of 3T3-L1 Fibroblasts to Adipocytes*, Journal of Lipid Research, 43:676-684, May 2002 (BPA exposure can trigger two main processes in developing obesity);
- K. Sakurai et al, *Bisphenol A Affects Glucose Transport in Mouse 3T3-F442A Adipocytes*, British Journal of Pharmacology, 141:209-214 (2004) (confirming findings of Masuno, showing that BPA increased uptake of sugar into fat cells);
- N.J. MacLusky, T. Hajszan, and C. Leranth, *The Environmental Estrogen Bisphenol A Inhibits Estradiol-Induced Hippocamopal Synaptogenesis*, Environmental Health Perspectives, 13:675-679 (2005) (in some areas of brain, BPA can inhibit activity of estrogen);
- W.E. Barlow et al, *Prospective Breast Cancer Risk Prediction Model for Women Undergoing Screening Mammography*, Journal of the National Cancer Institute, 17:Vol. 98, 6 September 2006 (BPA altered growth of mammary tissues to increase risk of breast cancer);

- M. Sakaue et al, *Bisphenol A Affects Spermatogenesis in the Adult Rat Even at a Low Dose*, Journal of Occupational Health, 43:185-190 (2001) (BPA reduced sperm count in rats even when exposure is after puberty, "... environmental endocrine disrupters such as BPA alter spermatogenesis in a linear manner in a dose range which is perhaps relevant to the daily level of exposure in man.")

35. In 2006, a group of 38 leading BPA scientists held a meeting sponsored by the National Institutes of Health to examine the relationship between BPA and the negative trends in human health observed in recent years, including increases in abnormal penile/urethra development, early sexual maturation in females, increased neuron-behavioral problems such as ADHD and autism, increased childhood and adult obesity and Type II diabetes, regional decreases in sperm count, and increases in hormonally-mediated concerns. These scientists concluded that extensive evidence documents that negative health outcomes may not manifest until long after BPA exposure during development takes place. Developmental effects are irreversible and result from low-dose exposures during brief sensitive periods in development even though BPA may not be detected when the health problem is expressed. Furthermore, the scientists' findings indicate that studies of acute effects of high doses, relied upon by industry to defend the use of BPA, are not particularly relevant in considering effects on humans. *See Chapel Hill Bisphenol A Expert Panel Consensus Statement: Integration of Mechanisms, Effects in Animals and Potential to Impact Human Health at Current Levels of Exposure* (Exhibit E).

36. The Environmental Working Group ("EWG"), a non-profit organization founded in 1993, which conducts environmental investigations about toxins and other issues, The EWG has called into serious question the objectivity and validity of work done to evaluate BPA risks

by groups sponsored by the National Institute of Environmental Health Sciences ("NIEHS"). The EWG has found the NIEHS research to be flawed by conflicts of interest, and responsible for the federal government's failure to adequately regulate BPA. *See* Letter from Anila Jacob, Senior Scientist for EWG, to Dr. Michael Shelby, Director of NIEHS Center for the Evaluation of Risks to Human Reproduction (Jan, 25, 2008) (critiquing results from NIEHS Bisphenol A (BPA) Expert Panel Report), *available at* http://www.ewg.org/files/BPAletter20080125.pdf (last visited June 4, 2008).

37. Similarly, U.S. Representative Henry A. Waxman and members of the House Committee on Oversight and Government Reform have been critical of the work of these same groups and of EPA's failure to test and control endocrine disrupting substances such as BPA. *See* Letter from Henry Waxman, Chair of House Committee on Oversight and Government Reform, and Barbara Boxer, Chair of Senate Environment and Public Works Committee, to David Schwartz, Director of NIEHS (Feb, 28, 2008) (requesting a briefing regarding allegations that NIEHS Bisphenol A (BPA) Expert Panel Report was biased), *available at* http://oversight.house.gov/documents/20070228174926-82628.pdf (last visited June 4, 2008).

38. On November 26, 2007, the National Toxicology Program's Center for the Evaluation of Risks to Human Reproduction released an Expert Panel Report on the Reproductive and Developmental Toxicity of BPA. That report, authored by a twelve-member independent panel made up of government and non-government scientists, expressed some concern that exposure of children to BPA causes neural and behavioral effects, and minimal concern that BPA exposure in children accelerates puberty. *See* Center for the Evaluation of Risks to Human Reproduction, *NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Bisphenol A*, Nov. 26, 2007, *available at*

http://cerhr.niehs.nih.gov/chemicals/bisphenol/BPAFinalEPVF112607.pdf (last visited June 4, 2008).

39. As referenced above, on April 14, 2008, the National Toxicology Program, a division of the National Institutes of Health, released a Draft NTP Brief on BPA, with a finding that BPA is potentially dangerous to human development and reproduction. Specifically, the Draft NTP Report concluded that the scientific evidence supports a finding of some concern for exposure to BPA in fetuses, infants and children, as laboratory animal studies have reported that low level exposure can cause changes in behavior, in the brain, prostate gland, mammary gland, and the age at which females attain puberty. In addition, the Draft NTP Brief noted that studies with laboratory animals have shown that exposure to high dose levels of BPA during pregnancy and lactation can reduce survival, birth weight, growth of offspring early in life, and delay the onset of puberty. *See* Exhibit A.

40. In 2007, the Milwaukee Journal Sentinel investigated the use of chemicals, in particular, endocrine disruptors, reviewing over 250 studies from the last 20 years, thousands of regulatory and industry documents, and interviewing over 100 experts. In its article, the newspaper exposed that although no action had been taken under a federal regulatory program developed for the testing of endocrine disruptors, such as BPA, millions of dollars had been spent on the program. In a follow up article addressing BPA specifically, the newspaper examined 258 studies, with the majority finding BPA to be harmful. The newspaper also noted that the National Toxicology Program Report discussed above is the first time a federal agency has expressed that BPA poses a potential harm to humans. *See* S. Rust et al., *Are Your Products Safe? You Can't Tell.* Milwaukee Journal Sentinel, Nov. 25, 2007; S. Rust et al., *WARNING: The*

Chemical Bisphenol A Has Been Known to Pose Severe Health Risks to Laboratory Animals. AND THE CHEMICAL IS IN YOU. Milwaukee Journal Sentinel, Dec. 2, 2007. (Exhibit F)

41. The Milwaukee Journal Sentinel articles were also the focal point of a recently televised PBS program discussing the harmful effects of BPA in humans. *See Exposé on Bill Moyers' Journal: Chemicals in Our Food* (PBS television broadcast May, 23, 2008), *available at* http://www.pbs.org/moyers/journal/05232008/profile.html) (transcript on file at http://www.pbs.org/moyers/journal/05232008/transcript2.html). (Exhibit G)

42. Following the release of the Draft NTP Report, Senator Charles Schumer of New York introduced bill S. 2928, known as the "BPA-Free Kids Act of 2008," on April 29, 2008. The bill seeks to ban any children's product that contains a detectable amount of BPA, which will be treated as a banned hazardous substance under the Federal Hazardous Substances Act. On May 20, 2008, Senator Schumer, along with Representatives Hilda Solis and Henry Waxman of California, introduced bill H.R. 6100, known as the "Kid Safe Chemicals Act" to further amend the Federal Hazardous Substances Act.

43. In addition, retailers have begun pledging to remove baby bottles containing BPA from their shelves. To date, Wal-Mart, Toys "R" Us, Babies "R" Us, REI, as well as Defendants NNIC and Playtex, have all issued statements announcing that they will phase out baby bottles and training cups containing BPA. Retailer Whole Foods Market, Inc. ceased selling these products entirely in early 2006.

44. On April 19, 2008, the Canadian Health Ministry released a Risk Management Scope for Phenol, 4.4' (methylethylidene)bis-(BPA), including a Draft Screening Assessment Report Conclusion. The Draft Report proposed that BPA is toxic to human health and the environment as defined in the Canadian Environmental Protection Act, and opened a sixty day

comment period to determine whether BPA meets the criteria under section 64 of CEPA 1999, such that the Canadian government will be in a position to move to prohibit the importation, sale and advertising of all polycarbonate baby bottles. *See* Environment Canada and Health Canada, *Draft Screening Assessment for Phenol, 4,4' -(1-methylethylidene)bis- (Bisphenol A)*, Chemical Abstracts Service Registry Number 80-05-7, April 2008, *available at* http://www.ec.gc.ca/substances/ese/eng/challenge/batch2/batch2_80-05-7_en.pdf (last visited June 4, 2008).

45. Meanwhile, Canadian retailers are pulling water bottles and food storage containers off the shelves because Health Canada, the Canadian equivalent of the United States' FDA, is considering labeling the chemical as a dangerous substance. Jacob Goldstein, *Worries Grow Over Bisphenol A in Plastics,* Wall Street Journal Health Blog, April 16, 2008, *available at* http://blogs.wsj.com/health/2008/04/16/worries-grow-over-bisphenol-a-in-plastics/?mod=WSJBlog&mod=WSJBlog (last visited June 4, 2008).

46. After the release of the American and Canadian reports, the Israel Health Ministry announced guidelines for safe use of baby bottles containing BPA on April 30, 2008. The Israel Health Ministry advised using only baby bottles that were without scratches and less than a year old, and warned not to microwave or pour boiling or very hot water into baby bottles for making beverages or hot foods, as that may cause BPA to leach from the plastic. *See* Judy Siegel-Itzkovich, *Health Ministry Issues Guidelines on Baby Bottles*, Jerusalem Post, April 30, 2008, http://www.jpost.com/servlet/Satellite?pagename=JPost%2FJPArticle%2FShowFull&cid=1208870534621 (last visited June 4, 2008).

B. DEFENDANTS MARKET AND SELL THE HAZARDOUS BABY PRODUCTS DESPITE THE DANGERS TO INFANTS AND YOUNG CHILDREN

47. Defendants' Hazardous Baby Products make no mention or disclosure of the real or potential dangers of BPA exposure. They include no warnings or information about BPA on the packaging or in the marketing of Hazardous Baby Products. In fact, from looking at the product packages created, marketed, and distributed by the Defendants, it is impossible for a parent consumer to determine whether an infant feeding product contains BPA. Defendants keep parents and consumers ignorant of the potential dangers of BPA exposure. Defendants should not be permitted to continue imposing risks on children and future generations while profiting from the ignorance of its customers. The risk of harm outweighs the utility of a highly dangerous and controversial chemical component in the manufacture of plastic baby bottles and cups.

48. Defendants engaged in long-term advertising campaigns designed to market Hazardous Baby Products to new parents and other caregivers. Defendants were aware that the advertising concealed information that was material to the caregivers' purchasing decision, and its marketing was false and deceptive in claiming its products to be safe and healthful.

49. Most Defendants claim that their products are uniquely designed to support the baby's health. For example, Defendant Avent claims its Airflex Natural Feeding bottle contains an "Airflex valve", which mimics "baby's natural feeding rhythm" and "can help reduce overeating and spit up" and colic. Defendant Gerber markets its brand with a "Gerber Start Healthy Stay Healthy" slogan and a claim that its products "support healthy growth and development." One line of products tout docosahexaenoic acid, a polyunsaturated omega-3 fatty acid ("DHA"),to help "support brain and eye development." Certainly Gerber knows that parents would avoid products that impair such development. Defendant Handi-Craft claims the Dr. Brown's Natural Flow bottle has a "unique internal vent" that helps reduce colic and also

reduces the build up of fluid in baby's ears. Defendant Playtex claims its First Sipster® cup is designed by a "feeding specialist" and that its product "help[s] proper oral development."

50. Defendants' packaging similarly touts the healthfulness of its Hazardous Baby Products. For example, Avent labels its BPA products as "Natural Feeding", and Handi-Craft labels its BPA products with the slogan "Dr. Brown's Natural Flow." However, these products have a serious defect which can impair a child's health - BPA - which is not even mentioned on the package. Certainly Defendants know that if parents were made aware of the hazards they would avoid products that pose serious risks to the growth and development of their young children.

51. Most Defendants claim that their products are "clinically-proven" to support the baby's health in some manner. For example, Defendant Avent states its Natural Feeding bottles, which contain BPA, are "Clinically Proven to Reduce Colic". Defendant Playtex states that one line of its BPA Baby Products are "Clinically Proven" to "Significantly Reduce[] Gas, Spit-up & Colic," to "Help[] Prevent Ear Infections" and "Reduce[] Air Ingestion." Consumers rightfully believe that they would be informed if other clinical trials indicated a serious product defect, such as that which exists in Defendants' BPA Baby Products, or that such defects would be fixed before the product is sold.

52. Defendants' marketing is also designed to create a trust relationship. For example:

- Defendant Avent claims that "Choosing Philips AVENT means you have the assurance of superior quality products, designed with you and your baby's needs in mind. Through extensive research and clinical trials, Avent products work effectively together to promote baby's well-being".

- Defendant Gerber, using the slogan "Anything for Baby", claims their "commitment to the well-being of babies carries over into everything [they] do." Defendant Gerber's "Graduates" line of training cups is "dedicated to helping you provide your little one with good nutrition." Defendant Gerber also claims to be "committed to promoting good nutrition and healthy habits for children" and to be "one of the most trusted names in baby food and baby care for four generations."
- Defendant Handi-Craft Company markets the Dr. Brown's Natural Flow bottle as a "Gold Medical Design Excellence Awards – 2000 Winner" and claims that the feeding bottle is "Physician Designed." Furthermore, by manufacturing and marketing a bottle that creates "positive pressure flow, just like breastfeeding," Handi-Craft specifically targets its "Dr. Brown's" products to breastfeeding parents whom it knows, or should know, are particularly sensitive to health concerns, and would not buy Hazardous Baby Products, thereby placing their children at risk for developmental, neural and reproductive problems.
- Defendant Playtex states that "We know there is nothing more important to you than your baby's development."

53. Upon information and belief, Defendants were aware of the scientific studies discussed above and have conducted their own independent studies that have confirmed the fact that their products leach BPA into food and beverages under normal, everyday use.

54. Defendants are aware that there are alternative means for manufacturing its baby products and now manufacture and market BPA-free products as part of their product line. Most Defendants have even begun to manufacture and promote alternative BPA-free products. Yet, to increase profits, Defendants Avent, Gerber, Handi-Craft and Playtex continue to manufacture

and market Hazardous Baby Products without full disclosure of their risks. In addition to its Hazardous Baby Products containing BPA, Handi-Craft now offers BPA-free baby bottles made of glass and polypropylene, for which they charge a significant premium. *See http://www.handi-craft.com/* (showing three types of baby bottles, last visited June 4, 2008). As of May 20, 2008, Amazon.com sells the Dr. Brown's brand polypropylene bottles for approximately $40 for a set of three while Dr. Brown's BPA-laced bottles are on sale, five bottles for $16.27. Playtex and Gerber offer baby bottles and training cups made of polypropylene and/or polyethylene, but continue to manufacture and sell their Hazardous Baby Products which contain BPA. NNIC plans to eliminate BPA products entirely and offers a training cup made of copolyester. *See http://www.nalgene-outdoor.com/technical/bpaInfo.html* (stating NNIC's intention to phase out its BPA products "in response to consumer demand for [BPA-free] products", last visted June 4, 2008).

55. Having held themselves out as trustworthy sources of safe and healthy products, Defendants had a duty to disclose facts regarding the health risks their products imposed. This included the fact that their Hazardous Baby Products contained BPA that would leach into food and beverages under normal, everyday use, and the serious health risks posed by such BPA-exposure..

56. Defendants have misrepresented the risks of harm associated with its Hazardous Baby Products and have failed to make honest disclosures to Plaintiff and other similarly situated consumers. Plaintiff, and others similarly situated, relied upon the Defendants' misrepresentations and lack of disclosure and have sustained injuries as a result thereof.

57. These studies confirm that Defendants' Hazardous Baby Products contain levels of BPA that are potentially unsafe and could threaten the health and safety of children, including

the children of and in the care of members of the Class. Thus, members of the Class reasonably may feel compelled to discontinue the use of such products causing them economic harm.

V. CLASS ACTION ALLEGATIONS

58. Plaintiff brings this Illinois State class action pursuant to Rule 23 of the Federal Rules of Civil Procedure. Plaintiff brings this action on behalf of herself and all members of a Class comprised of all persons who, during the period May 2003 through the present purchased and/or acquired the Hazardous Bottles, Cups, and Containers.

59. The Class is so numerous that joinder of all members is impracticable. Plaintiff believes that the total membership of the Class numbers in the thousands.

60. There are many common questions of law and fact involving and affecting the parties to be represented. These common questions of law or fact predominate over any questions affecting only individual members of the Class Common questions include, but are not limited to, the following:

a. Whether the Hazardous Baby Products are defective;

b. Whether Defendants negligently manufactured, distributed, marketed, tested and/or sold the Hazardous Baby Products;

c. Whether there is an increased risk of serious health problems as a result of the BPA in the Hazardous Baby Products;

d. Whether Defendants conducted adequate studies and quality tests of their Hazardous Baby Products to determine whether and to what extent the Hazardous Baby Products were contaminated with BPA or leached BPA following exposure to heat or liquids;

e. Whether Defendants engaged in deceptive and unfair business and trade practices;

f. Whether Defendants knowingly or negligently concealed or omitted material information concerning the safety of the Hazardous Baby Products;

g. Whether the Class is entitled to injunctive relief; and

h. Whether Defendants falsely and fraudulently misrepresented in their advertisements and promotional materials, and other materials the safety of the Hazardous Baby Products.

61. Plaintiff's claims are typical of the claims of the respective Class she seeks to represent, because Plaintiff and all members of the proposed Class have purchased Hazardous Baby Products and/or are at risk of serious health problems.

62. Plaintiff will fairly and adequately protect the interests of the Class, and has retained attorneys experienced in class actions and complex litigation as her counsel.

63. Defendants have acted or refused to act on grounds generally applicable to the Class, thereby making appropriate final injunctive relief.

64. Plaintiff avers that the prerequisites for class action treatment apply to this action and that questions of law or fact common to the Class predominate over any questions affecting only individual members and that class action treatment is superior to other available methods for the fair and efficient adjudication of the controversy which is the subject of this action. Plaintiff further states that the interests of judicial economy will be served by concentrating litigation concerning these claims in this Court, and that the management of this Class will not be difficult.

VI. COUNT ONE
BREACH OF IMPLIED WARRANTY

65. Plaintiff repeats and realleges each and every allegation contained above as if fully set forth herein.

66. The Uniform Commercial Code § 2-314, codified at 810 Ill. Comp. Stat. 5/2-314, provides that unless excluded or modified, a warranty that the goods shall be merchantable is implied in a contract for their sale if the seller is a merchant with respect to goods of that kind.

67. Defendants manufactured, marketed and sold the Hazardous Baby Products and represented that the Hazardous Baby Products were fit for use by children. Contrary to such representations, Defendants failed to disclose that the Hazardous Baby Products were defective as they contained BPA that could leach into liquids contained by the Hazardous Baby Products.

68. At all times, Illinois and the following 48 states listed below, including the District of Columbia, have codified and adopted the provisions of the Uniform Commercial Code governing the implied warranty of merchantability: Ala. Code § 7-2-314; Alaska Stat. §45.02.314; Ariz. Rev. Stat. Ann. § 47-2314; Ark. Code Ann. § 4-2-314; Cal. Com. Code § 2314; Colo. Rev. St § 4-2-314; Conn. Gen. Stat. Ann. § 42a-2-314; 6 Del. C. § 2-314; D.C. Code § 28:2-314; Fla. Stat. Ann. § 672.314; Ga. Code Ann. § 11-2-314; Haw. Rev. Stat. § 490:2-314; Idaho Code § 28-2-314; Ind. Code Ann. § 26-1-2-314; Iowa Code Ann. § 554.2314; Kan. Stat. Ann. § 84-2-314; Ky. Rev. Stat. Ann. § 355.2-314; La. Civ. Code Ann. art. § 2520; 11 Me. Rev. Stat. Ann. § 2-314; Md. Code Ann. § 2-314; Mass. Gen. Laws Ch. 106 § 2-314; Mich. Comp. Laws Ann. § 440.2314; Minn. Stat. Ann. § 336.2-314; Miss. Code Ann. § 75-2-314; Mo. Rev. Stat. § 400.2-314; Mont. Code Ann. § 30-2-314; Nev. Rev. Stat. U.C.C § 104.2314; N.H. Rev. Ann. § 382-A:2-314; N.J. Stat. Ann. § 12A:2-314; N.M. Stat. Ann. § 55-2-314; N.Y. U.C.C. Law §2-314; N.C. Gen. Stat. Ann. § 25-2-314; N.D. Stat § 41-02-314; Ohio Rev. Code Ann. § 1302.27; Okla. Stat. tit. 12A § 2-314; Or. Rev. Stat. § 72.3140; 13 Pa. Stat. Ann. § 2314; R.I. Gen. Laws § 6A-2-314; S.C. Code Ann. § 36-2-314; S.D. Stat. § 57A-2-314; Tenn. Code Ann. § 47-2-314; Tex. Bus. & Com. Code Ann. § 2-314; Utah Code Ann. § 70A-2-314; Va.

Code § 8.2-314; Vt. Stat. Ann. 9A § 2-314; W. Va. Code § 46-2-314; Wash. Rev. Code § 62A 2-314; Wis. Stat. Ann. § 402.314 and Wyo. Stat. § 34.1-2-314.

69. As designers, manufacturers, producers, marketers and sellers of Hazardous Baby Products, each Defendant is a "merchant" within the meaning of the various states' commercial codes governing the implied warranty of merchantability.

70. The Hazardous Baby Products are "goods," as defined in the various states' commercial codes governing the implied warranty of merchantability.

71. As merchants of the Hazardous Baby Products, Defendants knew that purchasers relied upon them to design, manufacture and sell baby feeding products that were reasonably safe and would not endanger their children's health.

72. Defendants designed, manufactured and sold Hazardous Baby Products to parents of young children and they knew that such products would be used by caregivers of young children to feed them.

73. At the time that Defendants designed, manufactured, sold and/or distributed the Hazardous Baby Products, Defendants knew the purpose for which the bottles, cups, and containers were intended and impliedly warranted that they were of merchantable quality; were free of hazardous substances; were free of manufacturing defects such as BPA contamination; were free of design defects; and were safe and fit for their ordinary purpose - as food or drink vessels for young children.

74. Defendants breached their implied warranties in connection with their sale of Hazardous Baby Products to Plaintiff and members of the Class. The Hazardous Baby Products were not safe and fit for their ordinary purpose and intended use as children's feeding products, and were not free of defects, such as BPA contamination which at low doses can lead to cancers,

impaired immune function, early onset of puberty, obesity, diabetes, and hyperactivity, among other problems.

75. As a direct and proximate result of Defendants' breach of implied warranties, Plaintiff and other members of the Class have been injured and have suffered damages, including, but not limited to the value of the Hazardous Baby Products had they been safe and fit for their ordinary purposes and an increased risk of serious health problems.

76. Plaintiff and the Class are entitled to judgment against the Defendants for actual damages in the form of restitution, litigation costs and attorneys fees.

VII. COUNT TWO
BREACH OF EXPRESS WARRANTY

77. Plaintiff repeats and alleges each and every allegation contained above as if fully set forth herein.

78. The Uniform Commercial Code § 2-313, codified at 810 Ill. Comp. Stat. 5/2-313 provides that an affirmation of fact or promise made by the seller to the buyer which relates to the good and becomes part of the basis of the bargain creates an express warranty that the goods shall conform to the promise.

79. As detailed above, Defendants manufactured, marketed and sold the Hazardous Baby Products and represented that the Hazardous Baby Products were safe and fit for use by children. Further, Defendants represented that the Hazardous Baby Products were free from hazardous substances including BPA.

80. Defendants expressly warranted that the Hazardous Baby Products were manufactured to conform with all safety requirements under U.S. federal and other applicable laws and regulations, industry developed standards, including the ASTM Standards, and product

specific standards, and that they were periodically reviewed and approved by independent safety testing laboratories.

81. The Hazardous Baby Products did not conform to these express representations because the Hazardous Baby Products were not safe for children, and were defective, because they contained BPA.

82. At all times, Illinois and the following 48 states listed below, including the District of Columbia have codified and adopted the provisions of the Uniform Commercial Code governing the express warranty of merchantability: Ala. Code 1975 § 7-2-313; Alaska Stat. § 45.02.313; Ariz. Rev. Stat. § 47-2313; Ark. Stat. § 4-2-313; Cal. Com. Code § 2313; Colo. Rev. Stat. Ann. § 4-2-313; Conn. Gen. Stat. Ann. § 42a-2-313; 6 Del. C. § 2-313; D.C. Stat. § 28:2-313; Fla. Stat. Ann. § 672.313; Ga. Code Ann. § 11-2-313; Haw. Rev. Stat. § 490:2-313; Idaho Code § 28-2-313; Ind. Code Ann. § 26-1-2-313; Iowa Code Ann. § 554.2313; Kan. Stat. Ann. § 84-2-313; Ky. Rev. Stat. Ann. § 355.2-313; 11 Me. Rev. Stat. Ann. § 2-313; Md. Code Ann. § 2-313; Mass. Gen. Laws. Ch. 106 § 2-313; Mich. Comp. Laws Ann. § 440.2.313; Minn. Stat. Ann. § 336.2-313; Miss. Code Ann. § 75-2-313; Mo. Rev. Stat. § 400.2-313; Mont. Code Ann. § 30-2-313; Nev. Rev. Stat. U.C.C § 104.2313; N.H. Rev. Ann. § 382-A:2-313; N.J. Stat. Ann. § 12A:2-313; N.M. Stat. Ann. § 55-2-313; N.Y. U.C.C. Law 2-313; N.C. Gen. Stat. Ann. § 25-2-313; N.D. Stat. § 41-02-313; Ohio Rev. Code Ann. § 1302.26; Okla. Stat. tit. 12A § 2-313; Or. Rev. Stat. § 72.3130; 13 Pa. Stat. Ann. § 2313; R.I. Gen. Laws § 6A-2-313; S.C. Code Ann. § 36-2-313; S.D. Stat. § 57A-2-313; Tenn. Code Ann. § 47-2-313; Tex. Bus. & Com. Code Ann. § 2-313; Utah Code Ann. §70A-2-313; Va. Code § 8.2-313; Vt. Stat. Ann. 9A § 2-313; Rev. Code Wash. Ann. § 62A.2-313; W. Va. Code § 46-2-313; Wis. Stat. Ann § 402.313; Wyo. Stat. § 34.1-2-313.

83. At the time that Defendants designed, manufactured, sold and/or distributed the Hazardous Baby Products, Defendants knew the purpose for which the Hazardous Baby Products were intended and expressly warranted that they were safe and fit for use by young children.

84. Plaintiff and other members of the Class relied upon the skill, superior knowledge and judgment of the Defendants to sell baby feeding products that were reasonably safe for use by young children and their caregivers. Until investigative journalists published recent studies by scientists questioning the safety of the products, Plaintiff could not have known about the risks associated with the Hazardous Baby Products. Plaintiff had no independent means of knowing that the baby feeding products were not safe or fit for their ordinary purpose and intended use, and were not free of manufacturing defects, but instead were potentially laden with BPA, which is a hazardous substance because of its danger to young children.

85. Defendants breached their express warranties in connection with the sale of the Hazardous Baby Products to Plaintiff and other members of the Class.

86. As a direct and proximate result of Defendants' breach of express warranties, Plaintiff and other members of the Class have suffered damages, including, but not limited to, an increased risk of serious health problems and the value of the Hazardous Baby Products had they been safe and fit for their ordinary purposes.

87. Plaintiff and the Class are entitled to judgment against the Defendants for actual damages in the form of restitution, litigation costs and attorneys fees.

VIII. COUNT THREE
CONSUMER FRAUD AND DECEPTIVE BUSINESS PRACTICES ACT

88. Plaintiff incorporates by reference all previous paragraphs as if fully alleged herein.

89. Defendants' concealment, misbranding and non-disclosure of BPA as alleged herein constitutes unlawful, deceptive and unfair business acts within the meaning of the Illinois Consumer Fraud and Deceptive Business Practices Act ("the Act"), 815 Ill. Comp. Stat. 505/1 *et seq.*, and similar statutory enactments of other states (including consumer protection and consumer sales practices acts).

90. Defendants have also violated the Act because in their commercial and business practices, they have used and/or employed practices prohibited by the Uniform Deceptive Trade Practices Act, 815 Ill. Comp. Stat. 510/2. Specifically, Defendants have: (1) represented that their Hazardous Baby Products are of a particular standard, quality or grade when they are of another; (2) advertised their Hazardous Baby Products with intent not to sell them as advertised; and/or (3) engaged in conduct which similarly creates the likelihood for confusion and misunderstanding.

91. Defendants engaged in unfair and unlawful conduct to profit from sales of products containing BPA and acquired money or property as a result of these unlawful practices.

92. Defendants' use of deceptive and unfair business acts or practices in marketing and/or selling their Hazardous Baby Products violates public policy and is substantially unethical, oppressive and injurious to consumers.

93. In particular, Defendants' statements as to the safety and the healthfulness of their Hazardous Baby Products and their concealment and non-disclosure of the presence of BPA in its Hazardous Baby Products are unfair and deceptive and have the capacity to confuse, mislead or deceive consumers and members of the public. Such practice occurred in the conduct of trade or commerce; it affected the public interest; and such practice proximately caused injury to Plaintiff and members of the Class in their business and/or property.

94. Defendants knowingly concealed, suppressed and/or failed to disclose material facts with the intent that consumers would rely upon such concealment, misbranding, suppression or non-disclosure.

95. Defendants' concealment, misbranding, suppression and non-disclosure and other acts described above continue to this day and present a threat to Plaintiff and members of the Class. Defendants' conduct also affects and threatens the public interest in other ways now unknown, but to be proven at trial.

96. As a result of Defendants' concealment, misbranding, suppression and non-disclosure, Plaintiff and Class members have been harmed and continue to be harmed.

97. Plaintiff and the Class are entitled to an injunction against Defendants' misleading and deceptive practices and a declaration that Defendants' actions constitute a violation of the consumer protection laws. In addition, Plaintiff and the Class are also entitled to judgment for actual damages sustained, as a result of Defendants' unfair and deceptive acts and practices, in the form of restitution, and reimbursements of litigation costs and attorneys fees.

IX. COUNT FOUR
STRICT PRODUCTS LIABILITY – DEFECT IN DESIGN OR MANUFACTURE

98. Plaintiff incorporates by reference all previous paragraphs as if fully alleged herein.

99. Defendants, as commercial suppliers of products containing BPA, have a duty to refrain from placing unreasonably dangerous products into the stream of commerce that are not fit for consumption or use which can cause injury to persons or property.

100. Defendants breached that duty, and continue to breach that duty, by placing unreasonably dangerous products into the stream of commerce that are not fit for consumption or use which can cause injury to persons or property. Defendants' Hazardous Baby Products were

unreasonably dangerous because they contained, and continue to contain, manufacturing and/or design defects.

101. The unreasonably dangerous products Defendants placed into the stream of commerce reached consumers, including Plaintiff and the Class, without substantial changes in the condition in which they were supplied.

102. Plaintiff and the Class were reasonably foreseeable users of Defendants' unreasonably dangerous products and used the unreasonably dangerous products in a foreseeable manner. As a result, Plaintiff and the Class have suffered significant damages which were caused directly and proximately by their use of Defendants' Hazardous Baby Products.

103. Plaintiff and the Class are entitled to judgment against Defendants for their actual damages in the form of restitution, and reimbursement of litigation costs and attorneys fees.

X. COUNT FIVE
STRICT PRODUCTS LIABILITY – FAILURE TO WARN

104. Plaintiff incorporates by reference all previous paragraphs as if fully alleged herein.

105. Defendants have placed unreasonably dangerous products that are not fit for consumption or use into the stream of commerce and which can cause injury to persons or property.

106. Defendants have also failed to warn reasonably foreseeable users of these unreasonably dangerous products, including Plaintiff and the Class, of the known dangers associated with these products despite the fact that Defendants knew, or should have known, of the dangers associated with their unreasonably dangerous products. Even after Defendants became aware, or should have become aware, of the dangerous conditions of their products, they failed to investigate potential problems in a reasonable manner and failed to warn consumers of

potential dangers, thereby allowing countless consumers to purchase the unreasonably dangerous products.

107. As a direct and proximate result of Defendants' actions, Plaintiff and the Class suffered significant damages.

108. Plaintiff and the Class are entitled to judgment against Defendants for their actual damages in the form of restitution, and reimbursement of litigation costs and attorneys fees.

XI. COUNT SIX
BREACH OF CONTRACT

109. Plaintiff incorporates by reference all previous paragraphs as if fully alleged herein.

110. Plaintiff and each member of the Class purchased Hazardous Baby Products from Defendants. A contract was created between Defendants and Plaintiff and each and every member of the Class.

111. By reason of the conduct described above, Defendants have uniformly breached their contracts with Plaintiff and members of the Class by: (a) failing to provide baby products that could safely be used by babies and infants; (b) failing to disclose that the goods purchased were not what Defendants represented them to be; (c) failing to act in good faith; (d) breaching warranties existing because of the contracts; and (e) such other actions now unknown but to be proven at trial.

112. As a proximate result of the aforementioned wrongful conduct and breach committed by Defendants, Plaintiff and members of the Class have suffered and will continue to suffer damages and economic loss in an amount to be proven at trial.

XII. COUNT SEVEN
UNJUST ENRICHMENT

113. Plaintiff repeats and realleges each and every allegation contained above as if fully set forth herein.

114. At all times relevant hereto, Defendants designed, manufactured, produced, marketed and/or sold Hazardous Baby Products that contained BPA. BPA is a developmental, neural, and reproductive toxicant that scientists have linked at very low doses of exposure to cancers, impaired immune function, early onset of puberty, obesity, diabetes, and hyperactivity, among other problems.

115. Plaintiff and members of the Class conferred upon Defendants benefits that were non-gratuitous without knowledge that the Hazardous Baby Products they purchased and used have exposed their young children to potential adverse health effects resulting from repeated exposure to BPA. Defendants accepted or retained the non-gratuitous benefits conferred by Plaintiff and members of the Class, with full knowledge and awareness that, as a result of Defendants' unconscionable wrongdoing, Plaintiff and members of the Class were not receiving products of high quality, nature, fitness or value that had been represented by Defendants and reasonable consumers would have expected. Retaining the non-gratuitous benefits conferred upon Defendants by Plaintiff and members of the Class under these circumstances made Defendants' retention of the non-gratuitous benefits unjust and inequitable. Because Defendants' retention of the non-gratuitous benefits conferred by Plaintiff and members of the Class is unjust and inequitable, Plaintiff and members of the Class are entitled to, and hereby seek disgorgement and restitution of Defendants' wrongful profits, revenue, and benefits in a manner established by the Court.

XIII. COUNT EIGHT
FRAUDULENT MISREPRESENTATION

116. Plaintiff incorporates by reference all previous paragraphs as if fully alleged herein.

117. Defendants, with knowledge and/or belief of the falsity of their statements, misrepresented and concealed from Plaintiff, the Class and consumers, the true nature of their Hazardous Baby Products.

118. These representations were knowingly made to Plaintiff, potential customers and the general public through uniform misbranding, concealment and non-disclosure, through mass media and point-of-sale advertising, and through other information prepared or disseminated by Defendants. Defendants at all times knew that Plaintiff and Class relied upon the labeling and lack of labeling provided by Defendants. Defendants' concealment, misbranding and non-disclosure were intended to influence consumers' purchasing decisions and were done with intentional disregard for the rights of consumers.

119. As a direct and proximate result of these misrepresentations, omissions and concealments, Plaintiff and the Class have been damaged in an amount to be proven at trial.

XIV. COUNT NINE
NEGLIGENT MISREPRESENTATION

120. Plaintiff incorporates by reference all previous paragraphs as if fully alleged herein.

121. Defendants have a duty to communicate accurate information to Plaintiff.

122. Defendants breached that duty, and continue to breach that duty, in their failure to disclose the potential risks associated with the use of their Hazardous Baby Products and/or

accurately disclose available research discussing BPA containing products, despite awareness that information is available.

123. Defendants negligently and/or recklessly misrepresented and concealed from the Plaintiff, the Class and consumers, the true nature of their Hazardous Baby Products.

124. These representations were negligently or recklessly made to Plaintiff, potential customers and the general public through uniform misbranding, concealment and non-disclosure, through mass media and point-of-sale advertising, and through other information prepared or disseminated by Defendants. Defendants at all times knew that Plaintiff and Class relied upon the labeling and lack of labeling provided by Defendants. Defendants' concealment, misbranding and nondisclosure were intended to influence consumers' purchasing decisions and were done with reckless disregard for the rights of consumers.

125. As a direct and proximate result of Defendants breaching this duty, and their misrepresentations, omissions and concealments, Plaintiff and Class members have been damaged in an amount to be proven at trial.

XV. COUNT TEN
DECLARATORY AND INJUNCTIVE RELIEF

126. Plaintiff incorporates by reference all previous paragraphs as if fully alleged herein.

127. Plaintiff and Class members are entitled to declaratory relief establishing that Defendants are strictly liable for design and manufacturing defects and failure to warn, have engaged in unfair and deceptive practices, and that their conduct constitutes negligent misrepresentation and concealment, breach of contract and warranty, and that Defendants were thereby unjustly enriched.

128. Plaintiff and Class members are entitled to injunctive relief forcing Defendants to permanently halt the wrongful conduct asserted herein, and remedying past concealment and non-disclosure with new disclosures and other measures.

XVI. PRAYER FOR RELIEF

WHEREFORE, Plaintiff, on her own behalf and on behalf of the Class, prays for judgment against Defendants as follows:

1. An order certifying that this action, involving Plantiff and the Class against Defendants Avent, Gerber, Handi-Craft, NNIC and Playtex, be maintained as a nationwide class action under Rule 23 of the Federal Rules of Civil Procedure and appointing Plaintiff and her undersigned counsel to represent the Class;

2. For economic, compensatory, and general damages on behalf of all members of the Class;

3. For an award of actual damages in the form of restitution;

4. For disgorgement of ill gotten gains as set forth herein;

5. For declaratory and injunctive relief as set forth herein;

6. For reasonable attorneys' fees and reimbursement of all costs for the prosecution of this action, based upon the creation of a common fund recovery and under the consumer protection act, and based upon other theories and statutory bases.

7. For such other and further relief as this Court deems just and appropriate.

JURY TRIAL DEMANDED

Plaintiff hereby demands a trial by jury on all issues so triable.

DATED this 12th day of June, 2008.

KELLER ROHRBACK LLP.

By: /s/ Lynn Lincoln Sarko

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08CV3407
JUDGE HOLDERMAN
MAGISTRATE JUDGE COX

TC

Exhibit A

DRAFT
NTP BRIEF ON BISPHENOL A

[CAS NO. 80-05-7]

April 14, 2008

Peer Review Date: June 11, 2008

NOTICE

This DRAFT NTP Brief is distributed solely for the purpose of public comment and pre-dissemination peer review. It should not be construed to represent final NTP determination or policy.

National Toxicology Program

National Institute of Environmental Health Sciences
National Institutes of Health
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

Table of Contents

Preface

The National Toxicology Program (NTP)[1] established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June 1998. The purpose of the CERHR is to provide timely, unbiased, scientifically sound evaluations of the potential for adverse effects on reproduction or development resulting from human exposures to substances in the environment. The NTP-CERHR is headquartered at the National Institute of Environmental Health Sciences (NIEHS) and Dr. Michael Shelby is the director[2]

CERHR broadly solicits nominations of chemicals for evaluation from the public and private sectors. Chemicals are selected for evaluation based upon several factors including the following:

- potential for human exposure from use and occurrence in the environment
- extent of public concern
- production volume
- extent of database on reproductive and developmental toxicity studies

CERHR follows a formal process for review and evaluation of nominated chemicals that includes multiple opportunities for public comment. Briefly, CERHR convenes a scientific expert panel that meets in a public forum to review, discuss, and evaluate the scientific literature on the selected chemical. Public comment is invited prior to and during the meeting. The expert panel produces a report on the chemical's reproductive and developmental toxicities and provides its opinion of the degree to which exposure to the chemical is hazardous to humans. The panel also identifies areas of uncertainty and where additional data are needed. Expert panel reports are made public and comments are solicited.

Next, CERHR prepares the NTP Brief. The goal of the NTP Brief is to provide the public, as well as government health, regulatory, and research agencies, with the NTP's conclusions regarding the potential for the chemical to adversely affect human reproductive health or children's development. CERHR then prepares the NTP-CERHR Monograph, which includes the NTP Brief, the Expert Panel Report, and public comments on that report. The NTP-CERHR monograph is made publicly available on the CERHR web site and in hardcopy or CD from CERHR.

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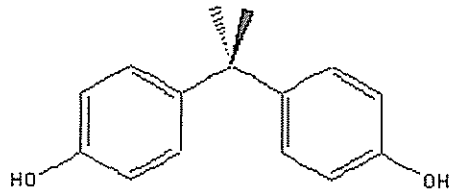
[1] NTP is an interagency program headquartered in Research Triangle Park, NC at the National Institute of Environmental Health Sciences, a component of the National Institutes of Health.

[2] Information about the CERHR is available on its web site (http://cerhr.niehs.nih.gov) or by contacting M.D. Shelby, Ph.D., Director, CERHR (P.O. Box 12233, MD EC-32, NIEHS, Research Triangle Park, NC 27709; telephone: 919-541-3455; facsimile: 919-316-4511; e-mail: shelby@niehs.nih.gov).

What is Bisphenol A?

Bisphenol A (BPA) is a chemical produced in large quantities for use primarily in the production of polycarbonate plastics and epoxy resins (Figure 1). It exists at room temperature as a white solid and has a mild "phenolic" or hospital odor. Polycarbonate plastics have many applications including use in certain food and drink packaging, e.g., water and infant bottles, compact discs, impact-resistant safety equipment, and medical devices. Polycarbonate plastics are typically clear and hard and marked with the recycle symbol "7" or may contain the letters "PC" near the recycle symbol. Polycarbonate plastic can also be blended with other materials to create molded parts for use in mobile phone housings, household items, and automobiles. Epoxy resins are used as lacquers to coat metal products such as food cans, bottle tops, and water supply pipes. Some polymers used in dental sealants or composites contain bisphenol A-derived materials. In 2004, the estimated production of bisphenol A in the United States was approximately 2.3 billion pounds, most of which was used in polycarbonate plastics and resins.

Figure 1. Chemical Structure of Bisphenol A ($C_{15}H_{16}O_2$; molecular weight 288.29)



CERHR selected bisphenol A for evaluation because it has received considerable attention in recent years due to widespread human exposures and concern for reproductive and developmental effects reported in laboratory animal studies. Bisphenol A is most commonly described as being "weakly" estrogenic; however, an emerging body of molecular and cellular studies indicate the potential for a number of additional biological activities. These range from interactions with cellular components that have unknown biological function to others that help mediate the actions of non-estrogenic hormones, such as androgens and thyroid hormones.

The NTP Brief on Bisphenol A is intended to be an environmental health resource for the public and regulatory and health agencies. It is not a quantitative risk assessment nor is it intended to supersede risk assessments conducted by regulatory agencies. The NTP Brief on Bisphenol A does not present a comprehensive review of the health-related literature or controversies related to this chemical. Only key issues and study findings considered most relevant for developing the NTP conclusions on concerns for potential reproductive and developmental human health effects of bisphenol A are discussed. Literature cited includes the most relevant studies reviewed in the CERHR Expert Panel Report on Bisphenol A and research articles published in the peer-reviewed literature subsequent to the deliberations of the expert panel.

Are People Exposed to Bisphenol A?[3]

[3] Answers to this and subsequent questions may be: *Yes, Probably, Possibly, Probably Not, No or Unknown*

Yes. The primary source of exposure to bisphenol A for most people is through the diet. While air, dust, and water (including skin contact during bathing and swimming) are other possible sources of exposure, bisphenol A in food and beverages accounts for the majority of daily human exposure [(*1*); reviewed in (*2, 3*)]. Bisphenol A can migrate into food from food and beverage containers with internal epoxy resin coatings and from consumer products made of polycarbonate plastic such as baby bottles, tableware, food containers, and water bottles. The degree to which bisphenol A migrates from polycarbonate containers into liquid appears to depend more on the temperature of the liquid than the age of the container, i.e., more migration with higher temperatures (*4*). Bisphenol A can also be found in breast milk (*5*). Short-term exposure can occur following application of certain dental sealants or composites made with bisphenol A-derived material such as bisphenol A-dimethyl acrylate (bis-DMA). Workers may be exposed during the manufacture of bisphenol A and bisphenol A-containing products.

Estimating human exposure to bisphenol A is generally done in one of two ways. Concentrations of bisphenol A can be measured directly in human blood, urine, breast milk, and other fluids or tissues ("biomonitoring"). Researchers can use biomonitoring information, such as the concentration of bisphenol A in urine, to estimate ("back calculate") a total intake that reflects all sources of exposure, both known and unknown. Scientists can also add, or aggregate, the amounts of bisphenol A detected in various sources, i.e., food and beverage, air, water, dust. The approach of aggregating exposure to estimate daily intake requires sources of exposure to be known and measured. In general, estimates based on biomonitoring are preferred for calculating total intake because all sources of exposure are integrated into the fluid or tissue measurement and do not have to be identified in advance. Estimates based on sources of exposure are useful to help discern the relative contributions of various exposure pathways to total intake.

The highest estimated daily intakes of bisphenol A in the general population occur in infants and children (Table 1). Infants and children have higher intakes of many widely detected environmental chemicals because they eat, drink, and breathe more than adults on a pound for pound basis. In addition, infants and children spend more time on the floor than adults and may engage in certain behaviors, such as dirt ingestion or mouthing of plastic items that can increase the potential for exposure.

Biomonitoring studies show that human exposure to bisphenol A is widespread (Table 2). The 2003-2004 National Health and Nutrition Examination Survey (NHANES III) conducted by the Centers for Disease Control and Prevention (CDC) found detectable levels of bisphenol A in 93% of 2517 urine samples from people 6 years and older (*6*). This study did not include children younger than 6 years of age. The CDC measured the "total" amount of bisphenol A in urine, a value that includes both bisphenol A and its metabolites. The CDC NHANES data are considered representative of exposures in the United States because of the large number of people included in the survey and the process used to select participants. In addition, the analytical techniques used by the CDC to measure bisphenol A are considered very accurate by the scientific community. Many smaller studies also report detection of bisphenol A in urine, blood, and other body fluids and tissues from people in the United States, Europe, and Asia [(*7-10*); studies published prior to mid-2007 are reviewed in (*2, 3, 11*)]. Concentrations of bisphenol A measured in breast milk and the blood of pregnant women in the United States are presented in Table 3.

It is helpful in interpreting the biomonitoring data for bisphenol A to understand how the body processes and excretes it once exposure occurs. Following ingestion, the majority of bisphenol A is quickly bound to glucuronic acid to produce bisphenol A-glucuronide, a metabolic process called glucuronidation that is carried out by enzymes primarily in the liver [reviewed in (*2*)]. Glucuronidation makes bisphenol A more soluble in water and, therefore, easier to eliminate in the urine and also minimizes its ability to interact with biological processes in the body. To a lesser extent, unconjugated parent (commonly referred to as "free") [4] bisphenol A is converted to other metabolites, primarily bisphenol A sulfate. Understanding the degree to which bisphenol A is metabolized is very important in determining whether bisphenol A poses a potential risk to human reproduction and development. While free bisphenol A and its major metabolites (bisphenol A-glucuronide and bisphenol A-sulfate) can all be measured in humans, only free bisphenol A is considered to be biologically active.

There is evidence in laboratory rodents that very young animals metabolize bisphenol A to its main biologically inactive metabolite, bisphenol A-glucuronide, less efficiently than adult animals (*12-14*). Neonatal rats do have some capacity to metabolize and eliminate bisphenol A; however, the enzyme systems that metabolize bisphenol A are not fully mature at this age and, as a result, neonatal rats have higher circulating concentrations of free bisphenol A in their blood compared to older animals given an equal exposure (*12*). There is also evidence for postnatal maturation of the corresponding enzymes in humans. Although a reduced ability or efficiency to glucuronidate is generally predicted for human fetuses and infants, this issue has not been specifically studied for bisphenol A [reviewed in (*2*)].

[4] Unmetabolized bisphenol A is commonly referred to as "free"; however, the majority of "free" bisphenol A circulating in human blood is bound to plasma proteins.

Table 1. Summary of Ranges of Estimated Daily Intakes in People Based on Sources of Exposure

Population	BPA µg/kg bw/day	Assumptions	References
Infant (0 – 6 months) Formula-fed	1 – 11*	• 1 assumes body weight of 4.5 kg and formula intake of 700 ml/day with 6.6 µg/L [maximum concentration detected in U.S. canned formula (*15, 16*)] (*2*) • 11 assumes body weight of 6.1 kg and formula intake of 1060 ml/day with (1) 50 µg/L bisphenol A/day migrating into formula from polycarbonate bottles (8.7 µg/kg bw/day); and (2) 14.3 µg bisphenol A/day ingested from powdered infant formula packed in food cans with epoxy linings (2.3 µg/kg bw/day) [0.143 kg powder/day (the amount of powder required to reconstitute a volume of formula of 1060 ml/day) containing 14.3 µg bisphenol A (100 µg bisphenol A/kg powder)]. 8.7 + 2.3 = 11 µg/kg bw/day (*17*)	(*2, 17-19*)
Infant (0 – 6 months) Breast-fed	0.2 - 1*	• 0.2 assumes body weight of 6.1 kg and breast milk intake of 1060 ml/day with 0.97 µg/L bisphenol A [maximum concentration of bisphenol A detected in Japanese breast milk samples (*20*)](*17*) • 1 assumes body weight of 4.5 kg and breast milk intake of 700 ml/day with 6.3 µg/L free bisphenol A [maximum concentration of free bisphenol A detected in U.S. breast milk samples (*5*)](*2*)	(*2, 17*)
Infant (6 – 12 months)	1.65 - 13*	• 1.65 assumes body weight of 8.8 kg with (1) 7 µg/L bisphenol A/day from formula intake of 700 ml/day with 10 µg/L (0.8 µg/kg bw/day); and (2) 7.6 µg/kg bisphenol A/day from ingestion of 0.38 kg canned food/day with 20 µg/kg (~0.85 µg/kg bw/day). 0.8 + 0.85 = 1.65 (*18*) • 13 assumes body weight of 7.8 kg, formula intake of 920 ml/day, and food consumption of 0.407 kg/day with (1) 50 µg/L bisphenol A migrating into formula from polycarbonate bottles (5.9 µg/kg bw/day); (2) 12.4 µg bisphenol A/day ingested from powdered infant formula packed in food cans with epoxy linings (1.6 µg/kg bw/day) [0.124 kg powder/day (the amount of powder required to reconstitute a volume of formula of 920ml/day) containing 12.4 µg bisphenol (100 µg bisphenol A/kg powder)]; (3) 40.7 µg bisphenol A/day ingested from canned food (5.2 µg/kg bw/day) [0.407 kg food/day containing 40.7 µg bisphenol A (100 µg bisphenol A/kg food)]; and (4) 2.04 µg bisphenol A/day migration from polycarbonate tableware (0.26, or ~ 0.3 µg/kg bw/day)[0.407 kg food/day containing 2.04 µg bisphenol A (5 µg bisphenol A/kg food)] 5.9 + 1.6 + 5.2 + 0.3 = 13.0 µg/kg bw/day (*17*)	(*16-19*)
Child (1.5 – 6 years)	0.043-14.7	• 0.043 is the mean (range: 0.018 – 0.071 µg/kg bw/day) based on individual body weight and measured concentrations of bisphenol in indoor and outdoor air, dust, soil, and liquid and solid food from daycare and home and the assumption of 100% absorption (*21*) • 14.7 assumes body weight of 14.5 kg and consumption of 2 kg canned food/day with (1) 200 µg bisphenol A/day ingested from canned food (~14 µg/kg bw/day) [2 kg food/day containing 200 µg bisphenol A (100 µg bisphenol A/kg food)]; and (2) 10 µg bisphenol A/day migration from polycarbonate tableware (~ 0.7 µg/kg bw/day)[2 kg food/day containing 10 µg bisphenol A (5 µg bisphenol A/kg food)] 14 + 0.7 = 14.7(*19*)	(*1, 17-19, 21, 22*)
Adult – general population	0.008 – 1.5**	• 0.008 assumes body weight of 74.8 kg and is based on measured concentrations of bisphenol A in 80 canned and bottled food items and a 24-hour dietary recall in ~4400 New Zealanders (*23*)	(*16-19, 22, 23*)

Population	BPA µg/kg bw/day	Assumptions	References
		• 1.5 assumes body weight of 60 kg and (1) 70 µg bisphenol A/day from canned food (1.2 µg/kg bw/day) [3 kg/day total consumption (1 kg solid food with 50 µg bisphenol A /kg and 2 L beverage with 10 µg bisphenol A /L)]; and 15 µg bisphenol A/day migration from polycarbonate tableware (0.25, or ~ 0.3 µg/kg bw/day)[3 kg food/day containing 15 µg bisphenol A (5 µg bisphenol A/kg food)] 1.2 + 0.3 = 1.5 µg/kg bw/day (*17*)	
Adult - occupational	0.043-100	• 0.043 is based on back calculating from a median urinary bisphenol A concentration of 1.06 µmol/mol creatinine (2.14 µg/g creatinine) from Hanaoka *et al.* (*24*). A daily intake of 0.043 µg/kg bw/day is based on the assumption of 1200 mg/day creatinine excretion (2.57 µg/day bisphenol excreted) and a body weight of 60 kg (*2*). • 100 is the maximal estimated exposures in U.S. powder paint workers based on time weighted averages of 0.001–1.063 mg/m^3, an inhalation factor of 0.29 m^3/kg day (*25*), 100% absorption from the respiratory system, and 8 hours worked per day (*2*).	(*2, 19, 25*)

*A study by Miyamoto *et al.* (*22*) reported much lower estimated intakes for infants (0.028 to 0.18 µg/kg bw/day); however, these estimates were excluded from the summary table because (1) insufficient detail was presented in the study to understand the assumptions used to derive these values, and (2) the authors assumed no bisphenol A in breast milk, an assumption not supported by data from the CDC (*5*) and Sun *et al.* (*20*).

** The European Union (*19*) calculated an extreme worst case scenario of ~ 9 µg/kg bw/day based on 1.4 µg/kg bw/day from food plus ~ 7 µg/kg bw/day from wine. The high estimated intake from wine (0.75 L wine/day with 650 µg bisphenol A /L = 325 µg bisphenol A/day, or ~7 µg/kg bw/day, from wine) was based on an extraction study conducted with an epoxy resin that is sometimes used to line wine vats. A study published subsequent to the evaluation by the European Union identified a maximum concentration of 2.1 µg bisphenol A /L in wine (*26*).

Table 2. Urinary Concentrations and Corresponding "Back Calculated" Daily Intakes of Bisphenol A in People (United States)

Population	Urinary Concentration of Total Bisphenol A (μg/L) median (25th – 95th percentile range)* (*6*)	Estimated Intake of Bisphenol A (μg/kg bw/day) median (25th – 95th percentile range)** (*27*)
All	2.7 (1.3 – 15.9)	0.0505 (0.0235 – 0.2742)
6-11 years	3.7 (1.7 – 16.0)	0.0674 (0.0310 – 0.3105)
12-19 years	4.2 (1.9 – 16.5)	0.0773 (0.0378 – 0.3476)
20-39 years	3.1 (1.5 – 15.4)	0.0563 (0.0272 – 0.2893)
40-59 years	2.4 (1.1 – 15.5)	0.0415 (0.0179 – 0.2335)
60+ years	1.9 (0.8 – 13.3)	0.0334 (0.0163 – 0.2331)
Female	2.4 (1.2 – 15.7)	0.0443 (0.0190 – 0.2705)
Male	3.2 (1.4 – 16.0)	0.0572 (0.0269 – 0.2778)

* The CDC data for ages 20-39 and 40-59 years were not presented in the study by Calafat *et al.* (*6*). Lakind *et al.* (*27*) obtained these values from data files available on the CDC website (http://www.cdc.gov/nchs/about/major/nhanes/nhanes2003-2004/lab03_04.htm). Lakind *et al.* (*27*) conducted a separate analysis of the CDC data and calculated mean and percentile values within 0.2 μg/L of those presented by Calafat *et al.* (*6*).

** Lakind *et al.* (*27*) assumed that daily intake of bisphenol A was equivalent to daily excretion. Daily excretion was calculated by multiplying the urine concentration of bisphenol A (μg/L) by 24-hour urinary output volume. Daily urinary volume was assumed to be 600 ml for children aged 6-11 years, 1200 for adult females, and 1600 for adult males. Body weight data from the 2003-2004 NHANES database was used to calculate daily intake adjusted for body weight.

Table 3. Blood and Breast Milk Biomonitoring of Bisphenol A in People (United States)

Biological Medium	Population (sample size)	Free BPA (μg/L) mean or median [range]	Total BPA (μg/L) mean or median [range]	Reference
Blood	Pregnant women (40)	mean: 5.9 [0.5 - 22.4]		(*10*)
Breast milk	Lactating women (20)	mean: 1.3; median: 0.4 [< 0.3 (LOD) - 6.3]	mean: 1.3; median: 1.1 [< 0.3 (LOD) - 7.3]	(*5*)

LOD = limit of detection

Can Bisphenol A Affect Human Development or Reproduction?

Possibly. Although there is no direct evidence that exposure of people to bisphenol A adversely affects reproduction or development, studies with laboratory rodents show that exposure to high dose levels of bisphenol A during pregnancy and/or lactation can reduce survival, birth weight, and growth of offspring early in life, and delay the onset of puberty in males and females. These effects were seen at the same dose levels that also produced some weight loss in pregnant animals ("dams"). The administered dose levels associated with delayed puberty (≥ 50 mg/kg bw/day), growth reductions (≥ 300 mg/kg bw/day), or survival (≥ 500 mg/kg bw/day) are far in excess of the highest estimated daily intakes of bisphenol A in children (< 0.0147 mg/kg bw/day), adults (< 0.0015 mg/kg bw/day), or workers (0.100 mg/kg bw/day) (Table 1). These "high" dose effects of bisphenol A are not considered scientifically controversial and provide *clear evidence* of adverse effects on development in laboratory animals.

In addition to effects on survival and growth seen at high dose levels of bisphenol A, a variety of effects related to neural and behavior alterations, precancerous lesions in the prostate and mammary glands, altered prostate gland and urinary tract development, and early onset of puberty in females have been reported in laboratory rodents exposed during development to much lower doses of bisphenol A (≥ 0.0024 mg/kg bw/day) that are more similar to human exposures. In contrast to the "high" dose developmental effects of bisphenol A, there is scientific controversy over the interpretation of the "low" dose findings. When considered together, the results of "low" dose studies of bisphenol A provide *limited evidence* for adverse effects on development in laboratory animals (see **Figures 2a & 2b**).

Recognizing the lack of data on the effects of bisphenol A in humans and despite the limitations in the evidence for "low" dose effects in laboratory animals discussed in more detail below, the possibility that bisphenol A may alter human development cannot be dismissed (see **Figure 3**).

Supporting Evidence

The NTP finds that there is clear evidence of adverse developmental effects at "high" doses of bisphenol A in the form of fetal death, decreased litter size, or decreased number of live pups per litter in rats (≥ 500 mg/kg bw/day) (*28, 29*) and mice (≥ 875 mg/kg bw/day) (*30-32*), reduced growth in rats (≥ 300 mg/kg bw/day) (*28, 29*) and mice (≥ 600 mg/kg bw/day) (*30, 31, 33*), and delayed puberty in male mice (600 mg/kg bw/day) (*33*), male rats (≥ 50 mg/kg bw/day) (*29, 34*) and female rats (≥50 mg/kg bw/day) (*29, 35*).

In addition to these "high" dose effects on survival and growth, the NTP recognizes that there are studies that provide evidence for a variety of effects at much lower dose levels of bisphenol A related to neural and behavioral alterations in rats and mice (≥ 0.010 mg/kg bw/day) (*36-42*), preneoplastic lesions in the prostate and mammary gland in rats (0.010 mg/kg bw/day and 0.0025 mg/kg bw/day, respectively) (*43-45*), altered prostate and urinary tract development in mice (0.010 mg/kg bw/day) (*46*), and early onset of puberty in female mice (0.0024 and 0.200 mg/kg bw/day) (*40, 47*).

These "low" dose findings in laboratory animals have proven to be controversial for a variety of reasons including concern for insufficient replication by independent investigators, questions on the suitability of various experimental approaches, relevance of the specific animal model used for evaluating potential human risks, and incomplete understanding or agreement on the potential adverse nature of reported effects. These issues have been extensively addressed elsewhere (*2, 48-52*) and were considered by the NTP when evaluating the bisphenol A literature.

How Was This Conclusion Reached?

Scientific decisions concerning health risks are generally based on what is known as the "weight-of-evidence." In the case of bisphenol A, evidence from the limited number of studies in humans exposed to bisphenol A is not sufficient to reach conclusions regarding possible developmental or reproductive hazard. In contrast, there is a large literature of laboratory animal studies. These include studies of traditional designs carried out to assess the toxicity of bisphenol A, as well as a wide variety of studies examining the possibility that exposure to "low" doses of bisphenol A, defined in the NTP Brief on Bisphenol A as ≤ 5 mg/kg bw/day (*53*), during critical periods of development might result in adverse health outcomes later in life due to its estrogenic or other biological properties. Many of these latter studies were designed not as toxicology studies but rather to probe very specific experimental questions and their results are not always easily interpreted with regard to how they contribute to the weight-of-evidence for human health risks.

Many of the laboratory animal studies of bisphenol A have technical or design shortcomings or their reports do not provide sufficient experimental details to permit an assessment of technical adequacy (*2*). As discussed in more detail below, the NTP did not establish strict criteria for determining which studies from the bisphenol A literature to consider for the evaluation. Rather, in an effort to glean information that might contribute to understanding the numerous reported effects of bisphenol A, NTP evaluated many individual study reports. Attention was paid to issues of sample size, control for litter effects, and various other aspects of experimental design; however, experimental findings were initially evaluated in relation to their biological plausibility and consistency across studies by multiple investigators. Studies were then evaluated as to their adequacy of experimental design and the likelihood that any inconsistent outcomes resulted from differences or shortcomings in experimental design. The NTP considered several overarching issues when evaluating the bisphenol A literature:

- Are the *in vivo* effects reproducible and/or biologically plausible?

Two issues become evident when considering the topic of reproducibility of effects in the bisphenol A literature. In some cases, the reproducibility of certain effects has been questioned because attempts at replication by other researchers using similar experimental designs did not necessarily produce consistent findings. This leads to reduced confidence in the utility of the effect for identifying a hazard. Numerous reasons have been suggested to explain the inconsistent findings including differences in sensitivity of the rodent model, i.e., species, strain, breeding stock, the author's funding source, the degree of laboratory expertise, and variations in diet,[5] animal husbandry, and route of administration. However, it is not known if these factors

[5] Understanding the impact of variations in dietary phytoestrogen content in laboratory animal studies of estrogenic compounds, including bisphenol A, is an active area of inquiry (*54*). Recent research suggests that bisphenol A may

account for the inconsistencies. In other cases, particularly for findings based on studies with very specific experimental questions, variations in experimental design are large enough to conclude that the reproducibility of the finding is essentially unknown. A number of these effects have not been addressed in traditional toxicity studies carried out to assess the toxicity of bisphenol A. Typically the safety studies do not probe for potential organ effects with the same degree of specificity or detail as those studies with specific experimental questions. The NTP evaluated the biological plausibility of findings with unknown reproducibility in light of supporting data at the mechanistic, cellular, or tissue level.

Another issue is that the "low" dose studies generally have not tested higher dose levels of bisphenol A, i.e., > 1 mg/kg. Testing over a wide range of dose levels is necessary to adequately characterize the dose-response relationship. Typically, effects are easier to interpret when the dose-response curve is monotonic and the incidence, severity, or magnitude of response increases as the dose level increases. Effects that have biphasic, or non-monotonic dose response curves, are well documented in toxicology, endocrinology and other scientific disciplines (*56, 57*), but can be more difficult to interpret, which often limits their impact in risk assessments or other health evaluations. Testing higher dose levels may also identify additional effects that aid in interpreting the "low" dose finding with respect to potential health risk.

- Do the *in vivo* effects represent adverse health findings in laboratory animals and/or humans?

A general limitation in the "low" dose literature for bisphenol A is that many studies have addressed very specific experimental questions and not necessarily established a clear linkage between the "low" dose finding and a subsequent adverse health impact. For example, when an effect is observed in fetal, neonatal, or pubertal animals, investigations may not have been conducted to determine if the effect persists or manifest as a clear health effect later in life. Establishing a linkage to an adverse health impact is important because many of the "low" dose findings can be described as subtle, which can make them difficult to utilize for risk assessment purposes. An additional factor in considering the adversity of a finding is determining if the experimental model is adequate for predicting potential human health outcomes.

- How should studies that use a non-oral route of administration be interpreted?

Because the majority of exposure to bisphenol A occurs through the diet (*1*), laboratory animal studies that use the oral route of administration are considered the most useful to assess potential effects in humans. However, a large number of the laboratory animal studies of bisphenol A have used a subcutaneous route of administration to deliver the chemical, either by injection or mini-pumps that are implanted under the skin. The consideration of these studies in health evaluations of bisphenol A has proven controversial (*2, 58*). There is scientific consensus that doses of bisphenol A administered orally and subcutaneously cannot be directly compared in adult laboratory animals because the rate of metabolism of bisphenol A differs following oral and non-oral administration. There is also consensus that fetal and neonatal rats do not metabolize bisphenol A as efficiently as adult rats at a given dose because the enzyme systems that are responsible for the metabolism of bisphenol A are not fully mature during fetal or neonatal life.

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alter DNA methylation (an epigenetic mechanism to alter phenotype) following exposure during development and that this effect may be offset by dietary exposure to methyl donors or the phytoestrogen genistein (*55*).

However, there is scientific debate on whether the reduced metabolic capability of neonatal rats is sufficient to adequately metabolize low doses of bisphenol A.

In adult rats and monkeys, bisphenol A is metabolized to its biologically inactive form, or glucuronidated, more quickly when administered orally than by a non-oral route, e.g., subcutaneously, intraperitoneally, or intravenously (*59-61*). This is because bisphenol A administered orally first passes from the intestine to the liver where it undergoes extensive conjugation primarily with glucuronic acid before reaching the systemic circulation ("first pass metabolism"). Because non-oral administration bypasses the liver, and therefore first pass metabolism, these routes of dosing in adult rats and monkeys result in higher circulating concentrations of biologically active, free bisphenol A compared to oral administration. Although not tested directly in adult laboratory mice, the impact of first pass metabolism is predicted to be similar. Thus, a subcutaneous dose is expected to have a greater biological effect than the same dose delivered by mouth in adult laboratory animals, including in the offspring of dams treated with bisphenol A during pregnancy.

Studies that administer bisphenol A through non-oral routes are most useful for human health evaluations when information on the fate, e.g., half-life, and concentration of free bisphenol A in the blood or other tissue is also available. For example, if the peak and average daily concentrations of free bisphenol A in blood were measured following non-oral administration, these values could then be compared to levels of free bisphenol measured in rodent studies where bisphenol A is administered orally or to levels measured in humans. However, none of the reproductive and developmental toxicity studies that treated animals by non-oral routes of administration determined the circulating levels of free bisphenol A or its metabolites. As a result, studies that treat laboratory animals using non-oral routes of administration have often been considered of no or of limited relevance for estimating potential risk to humans (*2, 19, 48*).

As discussed previously (see "Are People Exposed to Bisphenol A?"), fetal and neonatal rats do not metabolize bisphenol A as efficiently as the adult and, as a result, have higher circulating concentrations of free bisphenol A for some period of time compared to adults receiving the same dose (*12-14*). The peak concentrations of free bisphenol A in the blood of 4-day old male and female rat pups orally dosed with 10 mg/kg are 2013 and 162-times higher than the peak blood levels measured in male and female adult rats treated with the same mg/kg dose (*12*). A measure of how long it takes the body to eliminate free bisphenol A, referred to as "half-life," was also slower at this dose in neonatal rats: > 6.7 hours in male or female pups compared to well under an 1 hour in adult animals (*12*). Thus, for a given administered dose, blood levels of bisphenol A are higher in neonatal rats than in adults, and remain so longer following exposure. However, neonatal rats do have the ability to metabolize bisphenol A as indicated by the presence of bisphenol A glucuronide in the blood and the inability to detect the free form within the measurement sensitivity of the assay by 12 to 24-hours after treatment in females and males respectively (*12*).

Neonatal rats appear to be able to more efficiently metabolize bisphenol A when given at lower dose levels than at higher dose levels. Although Domoradzki *et al.* (*12*) also treated neonatal and adult animals with a lower dose level of bisphenol A, 1 mg/kg, making a direct comparisons based on age at exposure was not possible at that dose because free bisphenol A was too low to

be quantified in the blood of adults. However, in 4-day old male and female rats treated with 1 mg/kg of bisphenol A, 98 – 100% of administered bisphenol A was detected as bisphenol A-glucuronide[6] compared to 71 – 82% at 10 mg/kg, i.e., a smaller proportion of administered bisphenol A is glucuronidated at 10 mg/kg compared to 1 mg/kg. This would be expected when the limited capacity of young animals to metabolize bisphenol A is overwhelmed by dose levels of the compound. These data suggest more efficient metabolism by neonatal rats at 1 mg/kg compared to 10 mg/kg and imply that the age at exposure differences described above may be less profound in the "low" dose range (≤ 5 mg/kg bw/day).

Taken together these data indicate that, compared to adults at a given dose, neonatal rats (and presumably mice) metabolize bisphenol A more slowly and suggest that differences in circulating levels of free bisphenol A arising from oral and subcutaneous routes of administration as a result of "first-pass metabolism" are reduced in fetal or infant animals compared to adults. This prediction is supported by a recent study that did not detect differences in the blood concentration of free bisphenol A as a function of route of administration (oral versus subcutaneous injection) in 3-day old female mice following treatment with either 0.035 or 0.395 mg/kg of bisphenol A (*58*).

While more research in this area is warranted, data from studies where bisphenol A was given by subcutaneous injection were considered as useful in the NTP evaluation as oral administration when treatment occurred during infancy when the capacity to metabolize bisphenol A is low. Studies in adult animals, including pregnant dams, that administered bisphenol A by subcutaneous injection or by a subcutaneous mini-pump were considered informative for identifying biological effects of bisphenol A but not for quantitatively comparing exposures in laboratory animals and humans.

- What is the impact of limitations in experimental design and how should studies with these limitations be interpreted?

The impact on study interpretation due to limitations in experimental design has been a significant point of discussion for bisphenol A, especially for the issues of (1) small sample size, (2) a lack of experimental or statistical control for litter effects, and (3) failure to use a positive control (*2, 62*).

In general, studies with larger sample sizes will have more power to detect an effect due to bisphenol A exposure than studies with small sample sizes. For this reason, "negative" results from small sample size studies are viewed with caution. On the other hand, "negative" results from studies with larger sample sizes are usually considered more credible (*63*). However, there is no single sample size that can be identified as appropriate for all endpoints. The ability to detect an effect is affected by the background incidence, e.g., tumor or malformation rates in control animals, variability of a particular endpoint, and the magnitude of the effect. A sample size of at least six may be reasonable for many endpoints with low or moderate degrees of variability, such as body weight, but could be insufficient to detect statistically significant differences in endpoints with a higher degree of variability such as hormone level or sperm

[6] Based on percentage of plasma area under the curve (AUC) for radioactivity that was bisphenol A glucuronide.

count, or that occur infrequently such as malformations or tumor formation. These factors can make consistent detection of relatively small changes especially difficult on endpoints that have a high degree of inherent variability.

Lack of statistical or experimental control for litter effects was perhaps the single most common technical shortcoming noted in the developmental toxicity studies evaluated by the CERHR Expert Panel for Bisphenol A (*2*). Adequate control for litter effects when littermates are used in an experiment is considered essential in developmental toxicology. In 2000, the NTP co-sponsored a workshop with the U.S. Environmental Protection Agency referred to as the "Low Dose Endocrine Disruptors Peer Review." As part of the peer review, a group of statisticians reanalyzed a number of "low" dose studies (*63*). Based on studies that used littermates, they determined that litter or dam effects were generally present such that pups within a litter were found to respond more similarly than pups from different litters. The overall conclusion on this issue was that "[f]ailure to adjust for litter effects (e.g., to regard littermates as independent observations and thus the individual pup as the experimental unit) can greatly exaggerate the statistical significance of experimental findings." Studies that did not adequately control for litter effects were given less weight in the NTP evaluation and were generally only used as supportive material.

The NTP concurs with the opinion of several scientific panels that positive control groups can be very useful to evaluate the sensitivity and performance of a given experimental model (*2, 52, 63*). However, the NTP does not consider use of a positive control to be a required study design component particularly in animal model systems that are well characterized regarding the background incidence of "effects" and their variability. For bisphenol A studies, potent estrogens, such as diethylstilbestrol, ethinyl estradiol, 17β-estradiol, and estradiol benzoate, are the most commonly used positive control chemicals given bisphenol A's historical classification as a weak estrogen. Failure to obtain predicted responses with these chemicals is generally interpreted as a "failed" experiment, perhaps reflecting the selection of a relatively insensitive animal or experimental model or insufficient chemical challenge. Studies where no responses are observed in the positive control group have generally contributed less weight to evaluations of bisphenol A (*2, 52*). The significance of a "failed" positive control for bisphenol A varies from endpoint to endpoint and reflected more negatively on a study in the NTP evaluation when the predicted effect on reproductive tissue or function was not observed at dose levels that should be sufficiently high to produce an effect.

Although potent estrogens are used as positive controls for bisphenol A, an increasing number of molecular or cell-based ("*in vitro*") studies suggest that interpreting the toxicological effects of bisphenol A solely within the context of their consistency with a classic estrogenic mechanism of action, or even as a selective estrogen receptor modulator (SERM),[7] is overly simplistic. In addition to binding to the nuclear estrogen receptors ERα and ERβ, bisphenol A interacts with a variety of other cellular targets [reviewed in (*2, 64*)] including binding to a non-classical membrane-bound form of the estrogen receptor (ncmER) (*65-67*), a recently identified orphan nuclear receptor called estrogen-related receptor gamma ERR-γ (*68-72*), a seven-transmembrane estrogen receptor called GPR30 (*73*), and the aryl hydrocarbon receptor (AhR) (*74, 75*).

[7] A selective estrogen receptor modulator, or SERM, is a compound that binds nuclear estrogen receptors and acts as an estrogen agonist in some tissues and as an estrogen antagonist in other tissues.

Several *in vitro* studies show that bisphenol A can act as an androgen receptor antagonist (*74, 76-82*) and is reportedly mitogenic in a human prostate carcinoma cell line through interactions with a mutant tumor-derived form of the androgen receptor (*83*). Bisphenol A also interacts with thyroid hormone receptors (TRs) and, based on *in vitro* studies, is reported to either inhibit TR-mediated transcription (*84*), inhibit the actions of triiodothyronine (T3) or its binding to TRs (*85, 86*); or stimulate cell proliferation in a thyroid hormone responsive cell line (*87*). One *in vivo* study suggests that bisphenol A acts as a selective TRβ antagonist (*88*). Bisphenol A may also inhibit activity of aromatase, the enzyme that converts testosterone to estradiol (*74, 89*).

The toxicological consequences of the non-nuclear estrogen receptor interactions identified so far are unclear. In some instances, the physiologic role of the receptor is unknown or not well characterized, i.e., ERR-γ, GPR30, which makes interpreting the consistency of the data impossible with respect to the implicated mechanism based on the cellular or molecular studies and the observed *in vivo* toxicology. However, even when the physiological effects are generally understood, e.g., AhR or AR binding, aromatase function, scientists can only speculate as to the possible *in vivo* impacts when multiple receptor or other cellular interactions are considered together. Nevertheless, the identification of a growing number of cellular targets for bisphenol A may help explain toxicological effects that are not considered estrogenic or predicted simply based on the lower potency of bisphenol A compared to estradiol. Effects mediated through the ncmER are of interest because of its role in regulating pancreatic hormone release and because bisphenol A has been shown to activate this receptor *in vitro* at a concentration of 1 nM, which is similar to the active concentration of the potent estrogen diethylstilbestrol (*65, 67*).

Human Studies

Only a very small number of studies have looked at associations between bisphenol A exposure and disorders of reproduction or developmental effects in humans [(*10, 90, 91*), studies prior to mid-2007 reviewed in (*2, 3*)]. The human studies have looked at the relationship between urine or blood concentrations of total or free bisphenol A and a variety of health measures including levels of certain hormones that help regulate reproduction (*24, 92*), markers of DNA damage (*93*), miscarriage (*94*), chromosomal defects in fetuses (*95*), fertility and obesity in women (*90, 96, 97*), effects on the tissue that lines the uterus ("endometrium") (*90, 98*), polycystic ovary syndrome (*92, 97*), and birth outcomes and length of gestation (*10, 91*).

In these studies, there are reports of associations between higher urine or blood concentrations of bisphenol A and lower levels of follicle-stimulating hormone in occupationally exposed men (*24*), higher levels of testosterone in men and women (*92, 97*), polycystic ovary syndrome (*92, 97*), recurrent miscarriage (*94*), and chromosomal defects in fetuses (*95*). In addition, one study reported that patients with endometrial cancer and complex endometrial hyperplasia had lower blood levels of bisphenol A than healthy women and women with simple endometrial hyperplasia (*98*). Bisphenol A was not associated with decreased birth weight or several other measures of birth outcome in two recent studies (*10, 91*). Drawing firm conclusions about potential reproductive or developmental effects of bisphenol A in humans from these studies is difficult because of factors such as small sample size, cross-sectional design, lack of large variations in exposure, or lack of adjustment for potential confounders. However, the NTP

Expert Panel on Bisphenol A (*2*) concluded that several studies collectively suggest hormonal effects of bisphenol A exposure (*24, 92, 97*) including one in occupationally exposed male workers likely exposed through multiple routes including inhalation (*24*).

The NTP concurs with findings of the recent evaluations (*2, 3*) that while these studies may suggest directions for future research, there is currently insufficient evidence to determine if bisphenol A causes or does not cause reproductive toxicity in exposed adults. There is also insufficient evidence in humans to determine if bisphenol A does or does not cause developmental toxicity when exposure occurs prenatally or during infancy and childhood.

Laboratory Animal Studies

In contrast to the limited literature evaluating possible effects of bisphenol A in humans, the scientific literature on the toxic effects of bisphenol A in laboratory animals is extensive and expanding. For example, between February 2007, the cut-off date for literature included in the CERHR Bisphenol A Expert Panel Report, and April 11, 2008, more than 400 new articles related to bisphenol A were identified by PubMed search. All new studies related to the potential reproductive and developmental effects of bisphenol A were considered during preparation of the draft NTP Brief on Bisphenol A. However, only those studies that were considered the most informative for developing NTP conclusions are cited in the Brief. In addition to the new literature cited, many key studies reviewed in the expert panel report are cited herein.

Reproductive Toxicity Studies

The reproductive toxicity studies of bisphenol A include assessment of fertility, sperm counts, estrous cycling, and growth or cellular damage in reproductive tissues. Reproductive toxicity can be studied in animals exposed during adulthood, during development, or both. Conclusions on reproductive toxicity presented in this section of the NTP Brief on Bisphenol A are limited to the assessment of fertility in laboratory animals, regardless of when exposure occurred, and other indicators of reproductive effects in animals exposed only during adulthood. Assessments of aspects of the reproductive system other than fertility in animals exposed during development are discussed under the headings of "High" Dose and "Low" Dose Developmental Toxicity Studies below.

Studies show that bisphenol A does not reduce fertility in laboratory animals exposed in adulthood and/or during developmental at dose levels up to 500 mg/kg bw/day in rats (*29, 99*). Fertility may be negatively impacted at higher dietary doses ($\geq$ 875 mg/kg bw/day) in mice exposed as adults as indicated by a decreased number of litters per breeding pair (*32*), although two multigenerational reproductive toxicity studies did not report effects on fertility in mice at doses up to 1669 – 1988 mg/kg bw/day (*31, 33*). There are occasional reports of decreased fertility in smaller sample size studies of rodents exposed to much lower dose levels of bisphenol A during adulthood, such as oral treatment with 0.025 and 0.100 mg/kg bw/day in male mice (*100*). In the Al-Hiyasat *et al.* study, decreased pregnancy rates and increased incidence of resorptions in untreated female mice were attributed to effects in treated adult males, i.e., reductions in the number of testicular or epididymal sperm and hypothesized impaired sperm quality. However, the magnitude of the impact on weight-corrected testicular or epididymal

sperm number, ~16 to 37%, is not generally considered severe enough to account for the observed pregnancy rate decrease of ~33 to 40%.[8]

At high oral dose levels, adult exposure to bisphenol A caused reproductive toxicity in the form of altered estrous cycling in female rats (≥ 600 mg/kg bw/day)[9] (*102*) and cellular effects on the testis of male rats (235 mg/kg bw/day) (*103*). In addition, more subtle effects on maternal behavior, i.e., decreased duration of licking and grooming of pups, are reported at a lower oral dose in treated adult female rats (0.04 mg/kg bw/day) (*104*).

"High" Dose Developmental Toxicity Studies (> 5 mg/kg bw/day)

Results from developmental toxicity studies in mice and rats show adverse effects on pup survival and growth following maternal exposure to dose levels of bisphenol A defined by the NTP as "high" (> 5 mg/kg bw/day). In rats, a ~ 20 - 36% decrease in the number of pups per litter is reported following maternal dosing with ≥ 500 mg/kg bw/day (*28, 29*). Increases in fetal death and post-implantation loss are seen in rats treated with 1000 mg/kg bw/day during pregnancy (*28*). Reductions in fetal weight or growth during postnatal life occur at oral dose levels of ≥ 300 mg/kg bw/day in rats (*28, 29*). In mice, developmental toxicity is generally reported at higher oral doses in the form of fetal death, decreased number of live pups, reduced fetal or pup body weight at ≥875 mg/kg bw/day (*30-32*), and reductions in body weight during postnatal life in the F1 generation (but not the F2 generation) at 600 mg/kg bw/day (*33*). Fetal death in mice has also been observed in a recent study that reported embryo lethality following subcutaneous dosing with 10 mg/kg bw/day bisphenol A to pregnant mice (*105*). Occasionally, decreases in pup survival have been reported at much lower oral dose levels, such as 0.0024 mg/kg bw/day in mice (*106*). However, this effect is not typically reported at oral doses in this range even in studies from the same laboratory using a similar dosing regimen and the same source of mice (*107*).

Delayed onset of puberty (assessed by day of vaginal opening) has been reported in the female offspring of rats orally treated with bisphenol A at 50 mg/kg bw/day during gestation (*35*) or 500 mg/kg bw/day during gestation and lactation (*29*). In the study by Tyl *et al.* (*29*), this effect has been attributed to a decrease in body weight also observed at that dose and has not necessarily been considered a direct developmental effect (*19*). However, decreased body weight was not observed in females at the dose where delayed vaginal opening was reported by Tinwell *et al.* (*35*). This high dose effect of delayed vaginal opening is not the predicted effect of exposure to an estrogenic compound. It is worth noting that Tinwell *et al.* (*35*) did not detect any difference in onset of puberty in female rats when age at first estrous assessed by vaginal smear was used as the marker of puberty. Other "high" dose studies report no effect on onset of puberty in female rats exposed during gestation and lactation at maternal oral doses ranging from 3.2 to ~1000 mg/kg bw/day (*108-110*). One "high" dose study reported an accelerated onset of puberty in female rats following subcutaneous injection of bisphenol A during early post-natal life at 105 and 427 mg/kg bw/day (*111*). Delayed puberty in male rats treated during development has also

[8] Sperm counts in laboratory rodents and rabbits generally have to be severely impacted to cause infertility. Rats may still be fertile with a 90% reduction in sperm count (*101*).

[9] Animals were treated with 1000 mg/kg bw/day for 1-week and then the dose was reduced to 600 mg/kg for 22-25 additional days.

been reported at oral doses of ≥ 50 mg/kg bw/day (*29, 34*). This effect was associated with decreased body weight in the study by Tyl *et al.* (*29*), but not in the study by Tan *et al.* (*34*). A delay in puberty of 1.8 days has also been reported in male mice at 600 mg/kg bw/day in a 2-generation reproductive toxicity study (*33*).

With the exception of a possible morphological alteration of the urethra (discussed below) (*46*), bisphenol A has not been shown to cause malformations, such as skeletal birth defects or abnormally shaped or absent organs, in rats or mice at oral doses up to 1000 and 1250 mg/kg bw/day, respectively (*28, 30*). An indication of a possible developmental delay, apparent delayed bone formation ("ossification"), was reported at an oral dose level of 1000 mg/kg bw/day (*28*). A more subtle effect, cellular changes in the liver, in developmentally exposed animals has been reported at ≥ 50 mg/kg bw/day (*33*).

"Low" Dose Developmental Effects in Laboratory Animals (≤ 5 mg/kg bw/day)

- Neural and Behavioral Alterations

The NTP concurs with the CERHR Expert Panel on Bisphenol A that there is a sufficiently consistent body of literature to suggest that perinatal or pubertal exposure to "low" doses of bisphenol A causes neural and behavioral alterations in rats and mice, especially related to the development of normal sex-based differences between males and females ("sexual dimorphisms" or "sexually dimorphic").

Research on the effects of bisphenol A on the brain and behavior does not have as long a history as the assessment of reproductive tissues, but is now an active area of study that has been growing quickly in the past few years. Currently, the literature is composed of a collection of findings based on behavioral assessments, morphometric and cell-based measurements of the brain of laboratory animals, and *in vitro* studies to identify molecular and cellular targets and mechanisms of action. From these studies themes are emerging that suggest exposures to bisphenol A can produce a loss or reduction of sexual dimorphisms in non-reproductive behaviors and in certain regions of the brain as well as effects on the dopaminergic system. Neural effects are also implicated from mechanistic studies that show bisphenol A can interfere with thyroid hormone signaling.

Sexual dimorphisms include differences in the size, cellular composition, or molecular expression patterns of specific regions or structures in the brain. The studies detecting bisphenol A-induced changes in sexually dimorphic brain structures generally report a reduction or loss of sexual dimorphisms, for example, in the locus ceruleus (a brain region involved in mediating responses to stress) (*112, 113*), and the bed nucleus of the stria terminalis (involved in regulating emotional behavior) (*114*). Similar effects are reported in some, but not all, studies (*115-117*) of the anteroventral periventricular nucleus, a brain region that provides input to gonadotropin-releasing hormone neurons involved in regulating ovulation. The lowest administered doses delivered to either pregnant dams or neonatal animals associated with these effects range from ~0.03 mg/kg bw/day (oral) (*113*), 0.000025 mg/kg bw/day (subcutaneous mini-pump) (*116*) to ~100 mg/kg bw/day (subcutaneous injection) (*115*). Changes are not reported for all sexually dimorphic structures. One well-known sexually dimorphic structure reportedly not affected even

at doses up to 320 mg/kg bw/day in rats is the sexually dimorphic nucleus in the preoptic area (SDN-POA), a brain region that has a homologue in humans and is known to be modified by gonadal hormones during perinatal life (*108, 110, 112, 113, 117, 118*). Interpreting the potential human health or behavioral significance of effects on sexually-dimorphic brain regions can be difficult. For example, the bed nucleus of the stria terminalis is described as being responsive to reproductive hormones and generally involved in regulating emotional behavior (*119*), but the specific functions of this brain region in rats, and therefore the impact of loss of sexual dimorphism, remain unclear.

Effects on behavior have been assessed by a wide variety of experimental tests. Reported behavioral changes in rats or mice relate to play (*120*), maternal behavior (*36, 104*), aggression (*121, 122*), cognitive function (*123*), motor activity (*124, 125*), exploration (*38*), novelty-seeking (*38*) (*37, 126*), impulsivity (*126*), reward response (*37, 126-128*), pain response (*129*), anxiety and fear (*38, 40, 42, 130*), and social interactions (*131*). Many of these behaviors, including activity, anxiety, exploration, novelty seeking are sexually dimorphic to some degree. The lowest oral dose associated with behavioral changes is 0.01 mg/kg bw/day (via treatment to the pregnant dam) (*36-38*) and a number of behavioral changes have been reported following developmental exposure to oral doses between 0.01 and 1 mg/kg bw/day (*40, 42, 104, 120-123, 126, 129, 131-133*).

With the exception of a study that showed a slight increase in receptive behavior in females and an impairment of sexual performance in males (*121*), the loss of behavioral sexual dimorphisms do not relate to reproductive behavior (*108, 113, 134*). For instance, responses to novelty and exploratory behavior are sexually dimorphic behaviors where female mice tend to display more of these behaviors than males (*38, 126*). Bisphenol A seems to dampen this sex-difference by reducing the expression of these behaviors in female mice ("defeminization" or "masculinization") exposed during development, either through gestation via the dam with oral doses of 0.01 mg/kg bw/day or through gestation until weaning at 0.04 mg/kg bw/day (*38, 126*).

While a loss of sexual dimorphism seems to be one general trend observed in the behavior literature, findings for other effects can be more difficult to interpret. A number of studies have looked at the relationship between developmental exposure to bisphenol A and increased activity. The studies that most directly support an effect of increased activity administered bisphenol A directly into the brain (*124, 125, 135, 136*). This route of administration limits the ability to interpret these studies in relation to human exposure levels as well as to compare the findings to results from other studies that use more typical routes of administration. Other studies using similar behavior assessments have not reported differences in spontaneous motor activity in the offspring of dams orally treated with a range of doses from 0.1 – 400 mg/kg bw/day (*42, 137*). Indications of increased activity based on other types of behavioral tests are also mixed. Some studies report no impact of bisphenol A treatment on activity (*99, 133, 138*), increased morphine-induced locomotion in animals treated during development with bisphenol A (*127, 139*), no difference between control and bisphenol A treated animals in response to methylphenidate, a drug used to treat attention deficit hyperactivity disorder (ADHD) (*138*), and decreased amphetamine-induced activity in bisphenol A-treated male rats (*38*). The literature provides more consistent support for a loss of sexual dimorphism in locomotor activity. Bisphenol A exposure during development eliminated statistically significant sex differences

observed in control animals where females are more active than males (*113, 116*), or caused significant differences in activity consistent with a loss of sexual dimorphism, i.e., increased activity in male, but not female rats (*140*),

Certain behavioral effects such as alterations in locomotor activity, reward behavior, response to novelty, motivation, cognition, and attention can display some degree of sexual dimorphism but also implicate involvement of the dopaminergic system, a monoaminergic neurotransmitter. Interactions with the dopaminergic system are supported by findings that bisphenol A can alter the gene expression of D1, D3, and D4 dopamine receptors (*128, 136, 141*) and dopamine transporters (*136, 142, 143*). In addition, several studies report that perinatal exposure to bisphenol A can alter (usually decrease) expression of the rate limiting enzyme for dopamine synthesis, tyrosine hydroxylase (TH), that catalyzes the conversion of tyrosine to a pre-cursor of dopamine, dihydroxyphenylalanine (DOPA), in several regions of the brain including the substantia nigra (*136, 144*), the anteroventral periventricular nucleus of the hypothalamus (AVPV) (*115*), midbrain (*142*), limbic area (*143*), and rostral periventricular preoptic area (*116*).

Additional support for the brain as a target of bisphenol A is provided by a number of studies that report neural alterations at the cellular level including interactions with or changes in measures of expression of a number of receptors involved in brain function, such as estrogen receptors ERα and ERβ (*39, 145-147*), gamma-aminobutyric acid type A ($GABA_A$) (*148, 149*), progesterone (*150, 151*), aryl hydrocarbon receptor (AhR), retinoic acid receptor (RAR) alpha, retinoid X receptor (RXR) alpha (*152-154*), and thyroid receptors (*84-88*). Other studies report effects on neuronal migration or organization (*155, 156*), synaptogenesis (*157, 158*), GABA-induced currents (*149*), neuronal cell death (*159*), synaptic plasticity (*160*); thyroid receptor-mediated differentiation of oligodendrocytes (*161*), and reduced proliferation of neural progenitor cells (*162*).

The NTP concurs with the CERHR Expert Panel on Bisphenol A that the results of neurological and behavioral studies of exposures of laboratory animals to bisphenol A during development raise questions about possible risks to human development. The NTP also concurs that additional research is needed to more fully assess the functional, long-term impacts of exposures to bisphenol A on the developing brain and behavior. Overall, the current literature provides a collection of findings that cannot yet be easily interpreted for biological or experimental consistency or for relevance to human health. Part of the interpretive difficulty lies in reconciling findings of different studies that use different experimental designs and different specific behavioral tests to measure the same dimension of behavior.

- Mammary gland

There is evidence from rodent studies suggesting that perinatal exposure to bisphenol A via subcutaneous mini-pump at administered doses of 0.0025 to 1 mg/kg bw/day causes tissue changes ("lesions") in the mammary gland that may signal an increased susceptibility to develop mammary gland tumors later in life (*44, 45*). The evidence is not sufficient to conclude that bisphenol A is a rodent mammary gland carcinogen or that bisphenol A presents a breast cancer hazard to humans.

While bisphenol A has not been shown to cause cellular changes or cancer of the mammary gland in female rats and mice exposed as adults (*163*), two recent studies suggest that exposure of rats to bisphenol A during gestation may lead to the development of lesions in adulthood, ductal hyperplasia and carcinoma *in situ*, that may potentially progress to tumors, i.e., "preneoplastic" lesions (*44, 45*). In the study by Murray *et al.* (*45*) rats were treated with 0.0025 – 1 mg/kg bw/day bisphenol A during pregnancy by subcutaneous mini-pump. Significant increases in the incidence of hyperplastic ducts were reported in all dose groups of female offspring on post-natal day 50 and only in the lowest dose group of 0.0025 mg/kg bw/day on post-natal day 95 (sample sizes range from 4 – 6). A more severe lesion, carcinoma *in situ,* was present in female offspring in the 0.25 and 1 mg/kg bw/day groups on postnatal day 50 (25% incidence for both treatment groups) and postnatal day 95 (33% incidence for both treatment groups). These findings are supported by a study by Durando *et al.* (*44*)[10] where pregnant rats were treated with 0.025 mg/kg bw/day, again using a subcutaneous mini-pump. In this study, the percent of hyperplastic ducts was significantly increased in the female offspring at both postnatal days 110 and 180 (~2 – 5-fold). A non-significant increase in the incidence of ductal carcinoma *in situ* was noted following adult treatment with a subcarcinogenic dose of *N*-nitroso-*N*-methylurea, a chemical used in cancer research to assess susceptability to carcinogens (2/15 compared to 0/10 in control animals).

These findings are generally consistent with other reports of changes in mammary gland growth and development following perinatal exposure to bisphenol A that are related to an altered rate of maturation, e.g., advanced fat pad maturation, delayed lumen formation, enhanced duct growth, adoption of a pregnancy-like state, enhanced responsiveness to secondary estrogenic exposures, and potentially increased susceptibility to carcinogenesis, e.g., increased number or density of terminal end buds and ducts (*44, 45, 164-170*). Overall, these findings have been interpreted as indicating that developmental exposure to bisphenol A causes differential effects on maturation of epithelial and stromal elements in the breast tissue that may lead to a predisposition to disease onset later in life.

With the exception of an oral dosing study conducted by Moral *et al.* (*170*) that reported an increased number of mammary gland terminal ducts in the female offspring of rats treated during gestation with 0.250 mg/kg/day, the cellular and tissue-level effects on the mammary gland occurred following subcutaneous treatment via mini-pump with bisphenol A at doses of 0.000025 to 10 mg/kg/day (*44, 45, 164, 166-169*). The findings most closely linked to an "adverse" outcome, ductal hyperplasia and carcinoma *in situ*, were reported at 0.0025 – 1 mg/kg/day (*44, 45*).

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[10] The study by Durando *et al.* (*44*) implied that 99.9% DMSO was used in the mini-pump ["Pumps are designed to deliver 25 BPA (Sigma-Aldrich de Argentina S.A., Buenos Aires, Argentina) or only DMSO (99.9% molecular biology grade, Sigma-Aldrich de Argentina S.A.)"]. The manufacturer of the mini-pump does not recommend use of DMSO concentrations greater than 50% because it can degrade the pump reservoir material and potentially result in tissue inflammation and edema. For this reason, the CERHR Expert Panel on Bisphenol A considered this study critically flawed (*2*). The NTP concurs that use of a high concentration of DMSO is a technical short-coming, but is not convinced that this factor could account for the observed results. The NTP also considered the possibility that potential pump degradation could result in variations in administered dose, but concluded that the study was still useful to consider in the context of other findings.

Certain aspects of mammary gland cancer differ between rats and humans, e.g., metastases are uncommon in rodents, but the lesions identified in these two recent studies, ductal hyperplasia and carcinoma *in situ*, are generally recognized as intermediary steps in chemical-induced mammary gland cancer in the rat and as pre-neoplastic lesions in the human (*171-174*). The appearance of ductal hyperplasia and carcinoma *in situ* are similar enough between rats and humans that these findings in the rat are considered relevant to humans (*172*). In humans, a greater than mild degree of ductal hyperplasia and ductal carcinoma *in situ* are associated with increased relative risk of developing invasive breast carcinoma. It is important to note that the development of these lesions does not guarantee the formation of tumors or cancer in rats or humans and they are most appropriately interpreted as risk factors. If similar changes occur in women, the increased relative risks for developing invasive breast cancer range from 1.5 to 5-fold for moderate and atypical ductal hyperplasia and 8.0 to 10.0-fold for ductal carcinoma *in situ* (*175*). The relative risk is based on a comparison to women of the same age in the general population. For example, a 50-year old woman has a 1 in 39 chance of developing invasive breast cancer in the next 10 years. If a 50-year woman has atypical ductal hyperplasia, a form of ductal hyperplasia associated with a moderate level of increased relative risk (4 to 5-fold), then her chance of developing invasive breast cancer in the next 10 years increases to approximately 1 in 10 to 1 in 8.

The current literature is not sufficient to establish the reproducibility of the ductal lesion findings by multiple independent investigators. Bisphenol A was not shown to induce neoplastic or non-neoplastic lesions in the mammary gland of female rats (~74 and 135 mg/kg bw/day) or mice (650 and 1300 mg/kg bw/day) in two-year dietary cancer bioassays where exposure was initiated in young adult animals (5-weeks of age) (*163*). However, these studies did not include perinatal exposure and the NTP recognizes that adult-only exposure may not be sufficient to detect chemical carcinogens in hormonally-responsive tissues such as the mammary gland (*174*). Most of the toxicology studies of bisphenol A that included assessment of females following developmental exposure either (1) did not report examination of the mammary gland (*29, 35, 111, 176, 177*), or (2) collected mammary gland tissue but did not prepare the tissue in a manner that would readily reveal these changes, i.e., whole mounts (*33, 99*). The limited assessment of the mammary gland in these studies is critical because it is not clear that, if present, intraductal epithelial proliferations would have been detected during the routine histopathologic examinations. While more severe lesions, such as the presence of a mammary mass, would be detected during routine necropsy, the studies by Ema *et al.*, (*99*) and Tyl *et al.*, (*33*) were primarily designed to detect effects on reproduction and development and not tumor incidence. Animals were not followed-up for a sufficiently long period of time to necessarily expect to observe tumors in control animals or differences in tumor incidence between treatment groups. In both of these studies, mammary gland tissues in the parental (F0) and F1 generations of females were only examined after weaning of their pups and the animals would have been well under one year of age at the time of tissue collection.

The NTP concurs with recent reviews (*2, 178*) that additional data are needed to more completely understand the possible long-term consequences of disrupting mammary gland development in animals by bisphenol A exposure and its significance for human health. Namely, long-term follow-up studies with sufficient statistical power should be conducted to evaluate if the ductal hyperplasia and carcinoma *in situ* progress to mammary gland tumors, preferably

without the use of a secondary chemical challenge in adulthood. In addition, conducting the appropriate pharmacokinetic studies for the subcutaneous mini-pump would aid in interpreting the results. While researchers predict that circulating levels of total and free bisphenol A in the subcutaneous mini-pump studies would be quite low based on the administered dose (≤ 1 mg/kg bw/day), the lack of supporting pharmacokinetic information limits the ability to make comparisons to human exposures.

- Prostate and Urinary Tract

There is some evidence that perinatal exposure to bisphenol A in rodents may alter prostate and urinary tract development and predispose the prostate to develop hormonally-induced pre-neoplastic lesions later in life. The evidence is not sufficient to conclude that bisphenol A is a rodent prostate gland carcinogen or that bisphenol A presents a prostate cancer hazard to humans.

In mice, exposure of pregnant dams to bisphenol A at an oral dose of 0.010 mg/kg bw/day has been shown in one study to alter prostate development in offspring by increasing the number of prostatic ducts, ductal volume, and the proliferation of a cell population implicated in the development of prostate cancer (basal epithelial cells) in one or more regions of the prostate (*46*). This study also reported a urinary tract deformation where the urethra narrows near the neck of the bladder, an effect that, if permanent, could contribute to urine flow disorders. These effects were observed in fetal mice and it is unclear if they persist into adulthood or relate to a clear adverse health outcome. It is important to note that other studies have not reported severe consequences of urinary tract constriction in adult animals exposed during development that might be predicted based on the finding by Timms *et al.* including bladder stones, hydronephrosis, hydroureter, or other indications of kidney toxicity.

In Sprague-Dawley rats, subcutaneous injection of neonates with 0.010 mg/kg bisphenol A followed by adult hormone treatment[11] was reported to cause 100% of the animals to develop "low" grade (3/10 animals) or "high" grade (7/10 animals) prostate intraepithelial neoplasia (*43*).[12] The incidence of prostate intraepithelial neoplastic lesions in animals that did not receive the adult hormone treatment was not significantly different from controls (2/6 versus 1/9 in control animals). Proposed biological mechnisms to account for the effects of bisphenol A on the prostate include altered DNA methylation patterns in genes that help regulate prostate development and growth as an epigentic mode of action (*43, 180*). The use of adult hormone treatment to promote the development of prostate intraepithelial neoplasia lesions complicates

[11] Animals were given Silastic capsule implants packed with estradiol and testosterone that result in serum concentrations of ~75 pg/ml estradiol and 3 ng/ml testosterone. This hormone treatment is intended to mimic the ratio of estradiol to testosterone in the aging male.

[12] One other study assessed bisphenol A's ability to predispose the prostate to develop prostate intraepithelial neoplasia lesions and tumors (*179*). In this study, female F344 rats were orally dosed with 0.05, 7.5, 30, or 120 mg/kg bw/day of bisphenol A during pregnancy and lactation. In order to induce prostate lesions and tumors, male offspring were treated with a chemical carcinogen, 3,2'-dimethyl-4-aminobiphenyl (DMAB). No statistically significant changes in prostate intraepithelial neoplasia lesions or carcinomas were observed. Differences between this study and the report of Ho *et al.* may be related to age at exposure (fetal versus neonatal and fetal), rat strain (F344 versus Sprague–Dawley), carcinogenic insult (DMAB versus estradiol + testosterone), route of administration (subcutaneous versus oral to dams), or other factor such as animal husbandry and housing.

the interpretation of this study when considering its relevance to human bisphenol A exposure. However, as discussed in more detail below, rodents are normally resistent to developing prostate cancer and the use of hormone treatment, chemical treatment, or other alternative animal model to obtain a more sensitive rodent model is considered an acceptable and recommended strategy in prostate cancer research (*174*).

The findings of Ho *et al.* (*43*) are consistent with a recent report of increased expression of cytokeratin 10 (CK10), a cell-marker associated with squamous differentiation, in adult male offspring of pregnant mice orally treated with 0.020 mg/kg bw/day bisphenol A during gestation (*181*). Chronic exposure to high doses of potent estrogens, such as diethylstilbestrol, leads to squamous metaplasia of the prostate, a tissue change characterized by a multilayering of prostatic basal epithelial cells. Squamous metaplasia is associated with benign prostatic hyperplasia or long-term estrogen treatment in patients with benign or malignant prostatic disease. The induction of CK10 expression in basal epithelial cells is an early indicator of changes leading to estrogen-induced squamous metaplasia. While the long-term health consequences of such an alteration are unclear, prostatic basal epithelial cells are implicated in the initiation and early progression of prostate cancer due to their function in maintaining ductal integrity and regulating the differentiation of luminal epithelial cell differentiation (*182*). It is important to note that prostates in the Ogural *et al.* study appeared morphologically the same as control animals based on the staining technique normally used in pathology (hematoxylin and eosin, or H&E). A stain specific for squamous keratin was required to detect the change. Thus, it is unclear whether similar changes in basal epithelial cell phenotype were present in other studies that evaluated the prostate using only an H&E stain.

The NTP concurs with the CERHR Expert Panel on Bisphenol A and another recent evaluation (*2, 178*) that additional studies are needed to understand the effects of bisphenol A on the development of the prostate gland and urinary tract. Studies should attempt to confirm these findings and include longer periods of follow-up to understand the significance of the structural and cellular effects observed in fetuses and to clarify the relevance of prostate intraepithelial neoplastic lesions resulting from bisphenol A exposure to the development of prostate cancer in these animals. Future research to clarify the role of bisphenol A in the development of prostate cancer presents a scientific challenge. Unlike humans where prostate cancer is common, it is the most common non-skin cancer in American men (*183*), rodents rarely develop prostate cancer. Of the almost 4,550 rats and mice used as controls in NTP 2-year inhalation or feed studies conducted during the last decade, only 1 cancerous tumor and 17 benign tumors ("adenoma") of the prostate gland were detected (*183*). No substances, including bisphenol A (*163*), have been identified as causing prostate tumors in NTP studies (*174*). The NTP has long recognized the limits of the traditional rodent cancer bioassay for detecting chemical-induced prostate tumors and organized a workshop in May 2006 to address this issue (*174*). Suggested strategies to improve the sensitivity of rodent models for detecting prostate cancer included using alternative models, e.g., genetically modified, and/or initiating exposure in perinatal life. In addition, NTP workshop participants suggested a more detailed histopathologic evaluation of the prostate because the assessment of human carcinogenic potential may be better determined based on chemical-induced preneoplastic changes rather than tumor incidence.

During its evaluation of bisphenol A exposure and prostate development, the NTP also considered a number of studies in rats or mice that have detected increased prostate weight at low doses (*107, 184*) or failed to detect this effect (*29, 33, 35, 99, 108, 113, 179, 185-190*). Prostate weight effects have taken on a special significance in the controversy surrounding bisphenol A because elevated prostate weight was the first "low" dose finding reported in laboratory animals (*107*) and prompted numerous follow-up studies. Attempts to understand the basis for discordant findings has generated considerable scientific discussion and debate including their review at the NTP-EPA Low-Dose Peer Review workshop mentioned earlier (*62*). In brief, the NTP believes that the overall conclusions of the Bisphenol A Subpanel of the NTP Low-Dose Peer Review remain valid with respect to "low" dose effects on prostate weight, i.e., increased prostate weight cannot be considered a general or reproducible finding.

More importantly, it is not clear that prostate weight should continue to be considered a critical endpoint in risk evaluations of bisphenol A given the relative crudeness of this measure. Changes in organ weight may be useful to identify potential target tissues, but become less important when additional data relating to structural, cellular, or functional integrity are available. Prostate enlargment does not correlate with the development of prostate histopathology or cancer in rodents, and the evaluation of prostate weight without corresponding assessment of histopathologic changes is not considered useful for determining carcinogenic potential (*191*).

In addition, changes in prostate weight are not necessarily observed in the same bisphenol A studies that report prostatic cellular or tissue-level changes. For example, no effects on prostatic lobe weight were observed in studies that reported (1) increased incidence and susceptibility to develop prostate intraepithelial neoplastic lesions (*43*), (2) changes in the prostatic periductal stroma and decreases in androgen-receptor positive stromal cells and epithelial cells positive for prostatic acid phosphatase (PAS), an enzyme produced by the prostate that can be found in higher amounts in men with prostate cancer (*192*), and (3) increased expression of CK10 in adult mice exposed as fetuses to 0.020 mg/kg bw/day via treatment of the dam or during adulthood to high doses of bisphenol A (2 – 200 mg pellets implanted under the skin for 3-weeks) (*181*).

- Puberty

NTP concurs with the CERHR Expert Panel on Bisphenol A that limited data are available at low doses to suggest an effect of accelerating the onset of puberty in female mice. Early onset of puberty has been observed in offspring of CF-1 mice orally treated with 0.0024 mg/kg/day during gestation (*47*) or C57BL/6 mice orally dosed with 0.2 mg/kg/day during gestation and lactation (*40*). These findings are supported by another study that noted an early onset of puberty in female ICR/Jcl mice whose mothers were treated with 0.02 mg/kg bw/day bisphenol A during gestation by subcutaneous injection (*176*). Two studies reporting effects on mammary gland growth and differentiation in female offpsring of CD-1 mice treated with bisphenol A during pregnancy through a subcutaneous mini-pump are consistent with an impact of bisphenol A on timing of puberty [(*164, 167*), reviewed in (*193*)]. In humans, early onset of puberty in girls is associated with elevated risk of developing breast cancer, early bone age maturation, and psychosocial impacts that include influencing age at first sexual intercourse and increasing risk for certain adolescent risk behaviors (*194-196*). Depending on the magnitude of the finding, early onset of puberty in laboratory animals can be considered an "adverse" effect in

reproductive toxicology risk assessment (*194*). The magnitude of the acceleration in puberty reported in the mouse studies ranges from 1 to 4.5 days (*40, 47, 176*).

Other studies have reported no effects on the timing of puberty in female mice [CF-1(*185*) or CD-1 (*33, 165*)] whose dams were treated with "low" doses of bisphenol A delivered orally or by subcutaneous mini-pump during gestation or during gestation and lactation. It is unclear if the inability of these studies to reproduce the advanced onset of puberty finding was due to variations in mouse strain and stock, timing of exposure, diet, or other facets of experimental design. The most consistent difference between the "positive" and "negative" studies lies in the approach used to measure onset of puberty. Age at first estrus is the most accurate indicator of puberty in rodents. This occurs at the same time as vaginal opening in rats. However, in mice, vaginal opening does not correlate well with puberty and the first day of detecting cornified cells in a vaginal smear, a sign of first estrus, is used to indicate the onset of puberty (*197*). The studies by Ashby *et al.*, Markey, *et al.*, and Tyl *et al.*, (*33, 165, 185*) that did not detect an effect of bisphenol A relied on age at vaginal opening in mice rather than the use of vaginal smears to assess onset of puberty. An additional issue associated with interpreting the study by Ashby *et al.*, (*185*) is the finding of a significant 3.6 day delay in the age of vaginal opening in the diethylstilbestrol positive control group (0.0002 mg/kg bw/day) when compared to the vehicle control group. A delay in puberty is inconsistent with the predicted estrogenic effect of accelerated puberty in the diethylstilbestrol group.

Additional studies are needed to establish the reproducibility of the finding that bisphenol A causes early onset of puberty in female mice at very low doses. The study by Howdeshell *et al.*, (*47*) reported a ~ 2.5 day acceleration of puberty in female offspring of mice orally treated with 0.0024 mg/kg bw/day during pregnancy based on a measure that is not standard in toxicology (the interval between vaginal opening and first estrus). Using the more standard interval of days from birth to first estrus, Ryan *et al.* (*40*) found ~ 4.5 day acceleration in puberty in the female offspring of dams treated during gestation and lactation with an oral dose of 0.2 mg/kg bw/day, but no effect at 0.02 mg/kg bw/day. The study by Honma *et al.* (*176*) reported a ~1 day earlier onset of puberty in the offspring of mice treated with 0.02 mg/kg bw/day by subcutaneous injection during pregnancy. As discussed previously, doses delivered orally and by subcutaneous injection in adult animals, including pregnant dams, cannot be directly compared due to route of administration differences in the metabolism of bisphenol A.

The data in female rats are less compelling for a possible "low" dose effect on puberty. A finding of accelerated puberty has been reported in Wistar rats (*44*), but most of the "low" dose literature does not support an effect (*29, 35, 45, 99, 113, 198, 199*).

The effects of bisphenol A on puberty in rats at "high" doses are generally inconsistent with the "low" dose effects reported in the mouse studies by Howdeshell *et al.* (*47*), Ryan *et al.* (*40*), and Honma *et al.* (*176*). Only one study has reported an effect on puberty in the predicted direction, i.e., acceleration following subcutaneous treatment on postnatal days 0 to 9 (*111*). Other studies reported no effect (*108-110*) or a delay in puberty at ≥ 50 mg/kg bw/day (*29, 35*). Four of these studies used a positive control group (*35, 108, 110, 111*). In these studies, responses to potent estrogens based on age at vaginal opening ranged from no effect (*108*), to statistically significant small or moderate acceleration [1.7 days (*35*); 2.4 days (*111*); 3.6 days (*110*)].

An area of uncertainty in the assessment of puberty is reconciling the general absence of an effect at "low" doses in rats with the mouse studies that found early onset of puberty in females when puberty was assessed by age at first estrous. The differences in outcomes cannot be attributed to use of single insensitive strain or stock as a variety of rat models were used in the "negative" studies: Sprague-Dawley, Wistar, Wistar-Furth rats, Wistar-derived Alderley Park, CD, and Donryu. Moreover, three of the "negative" puberty studies reported other "low" dose effects (*45, 113, 198*). Based on an evaluation of two negative studies that included "low" dose treatment groups and that used a positive control compound (*35, 113*), there is some support for a conclusion that vaginal opening may not be a sensitive indicator of estrogenic response in all strains of rat or experimental designs. The study by Tinwell *et al.* (*35*) reported a relatively small acceleration in puberty, 1.7 days, in Wistar-derived Alderley Park rats treated with what is considered a high dose level of ethinyl estradiol (0.2/0.1 mg/kg bw/day orally to dams during pregnancy). In contrast, the study by Kubo *et al.* (*113*) reported a more profound acceleration in puberty of 5.9 days in female offspring of Wistar rats exposed to diethylstilbestrol (0.050 mg/L in drinking water) during pregnancy and lactation (*113*). Another observation made from the rat studies that used a positive control group is that larger impacts on puberty onset (> 3 days) were more likely to be observed in studies that exposed animals during gestation and lactation or lactation (*110, 111, 113*) compared to gestation only (*35*); although, the Kwon *et al.* study (*108*) does not fit this profile (no effect on puberty following oral treatment with 3.2 – 320 mg/kg/day during gestation and lactation).

In summary, additional research is needed to assess the robustness of altered puberty at dose levels in the very low µg/kg bw/day range in mice, i.e. 0.0024 mg/kg bw/day. Research directed towards understanding the apparent differences in response between rats and mice on this measure would also be valuable. This issue has implications not just for the evaluation of bisphenol A, but also for characterizing possible effects on puberty for other weakly estrogenic compounds.

- Other Effects Considered

A variety of other effects in laboratory animals have been linked to "low" dose bisphenol A exposure during development, including decreased sperm quantity or quality, obesity, disruption of meiosis, changes in reproductive hormone levels, or cellular effects in reproductive tissues. These effects had less impact in shaping NTP's conclusions on potential risks to humans from bisphenol A exposure than the developmental effects observed at "high" doses on survival and growth and the "low" dose effects on brain and behavior, mammary gland, prostate gland, and onset of puberty in females described above.

In some cases, the relationship between a specific cellular- or tissue-level finding and a potential health effect in the whole organism is unclear. This is because there is often uncertainty about the functional impact of a cellular or mechanistic finding, such as the altered level of a receptor protein or change in enzyme activity. For example, the potential health impact that may result from uterine changes characterized by altered ERα and ERβ expression and from an increase in the number and appearance of uterine epithelial cells is unclear (*200*).

In other cases, the literature is not sufficiently developed. Newbold *et al.* (*201*) recently described a number of morphological changes in the ovaries and uteri of 18-month old mice that had received subcutaneous injections of bisphenol A at doses of 10, 100, or 1000 μg/kg on days 1-5 of life. Increases in cystic ovaries and cystic endometrial hyperplasia were statistically significant in the 100 μg/kg dose group but not at 1000 μg/kg. Non-statistically significant increases in the incidence of a variety of other ovarian and uterine proliferative lesions and cysts were also reported. Replication of these findings and further study of the linkage of early and late occurring events will be important in establishing a better understanding of any long-term consequences of exposures of the developing organism to bisphenol A.

As mentioned earlier, NTP Briefs are not meant to serve as comprehensive reviews of the scientific literature. Only key study findings and issues that relate to NTP conclusions on concerns for potential reproductive and developmental health effects in humans are typically presented. However, three reported "low" dose health effects (obesity, decreased sperm count or quality, and abnormalities of meiosis) that ultimately had less impact in determining the NTP's conclusions are briefly discussed below in order to illustrate the interpretive challenges associated with this literature. Two of these examples, obesity and impacts on sperm, are used to demonstrate findings that are not reported consistently enough to be considered reproducible. The third example relates to abnormalities of meiosis and is presented to demonstrate that effects predicted from *in vitro* studies are not necessarily observed in the *in vivo* studies.

Obesity

There is currently insufficient evidence to conclude that bisphenol A exposure during development predisposes laboratory animals to develop obesity or metabolic diseases such as diabetes, later in life. Obesity and metabolic disruption has become a research focus for bisphenol A based on several reports of increased postnatal growth following "low" dose exposure during development and several *in vitro* and *in vivo* studies that report effects related to altered carbohydrate and lipid regulation.

The NTP concurs with the CERHR Expert Panel on Bisphenol A that the effects of bisphenol A on body weight at "low" doses are inconsistent (*2*). A number of studies in rats and mice report increases in post-natal growth following developmental exposure to bisphenol A at oral doses of 0.0024 – 1.2 mg/kg bw/day (*47, 146, 198, 202*) or a subcutaneous dose of 0.5 mg/kg bw/day (*166*). Other "low" dose (≤ 5 mg/kg bw/day) studies in rats and mice have either not detected any significant effect on body weight (*35, 40, 42, 44, 99, 108, 113, 189, 201, 203*) or reported growth reductions (*29, 107, 137, 176, 204*). Differences in study outcomes cannot easily be attributed to the use of a potentially insensitive rodent model or experimental protocol because several studies that did not detect any significant difference in body weight reported other effects at "low" dose levels (*40, 42, 44, 113, 204*). The bases for the inconsistent findings are unclear but may relate to factors such as diet and differences in experimental design or analysis.

The data are currently too limited to conclude that developmental exposure to bisphenol A causes diabetes or other metabolic disorders later in life. Two studies in laboratory animals have assessed endpoints related to carbohydrate or lipid regulation. In adult male mice, a single subcutaneous dose of 0.010 or 0.100 mg/kg bw/day bisphenol A caused decreased blood glucose

and increased plasma insulin (*205*). Additionally, increased pancreatic insulin content and insulin resistance was reported at 0.100 mg/kg bw/day (administered orally or by subcutaneous injection) after a slightly longer period of dosing (4-days) (*205*). A recent study by Miyawaki *et al.* (*202*) assessed a variety of endpoints related to carbohydrate and lipid regulation in 1-month old mice that were exposed through maternal treatment during gestation and lactation with 0.001 or 0.010 μg/ml bisphenol A in drinking water (~0.26 and 2.42 mg/kg bw/during gestation). Endpoints included body weight, adipose tissue weight, and blood concentrations of leptin, total cholesterol, triglycerides, non-esterified fatty acid and glucose. Body weight and total cholesterol were significantly increased in female offspring in both dose groups although adipose tissue weight and leptin levels were only significantly increased in the 1 μg/ml treatment group. Male offspring in the high dose of 10 μg/ml were significantly heavier and had increased adipose tissue weight. Leptin levels were not associated with either of these effects in males. Significantly increased triglycerides and non-esterified fatty acid and decreased glucose were observed in male offspring in the low dose group of 1 μg/m. Although this study addresses the hypothesis that developmental exposure to bisphenol A can affect carbohydrate and lipid metabolism in postnatal life, the inconsistent pattern of effects on serum lipid levels, leptin, and glucose and lack of control for litter effects[13] makes the study on its own insufficient to draw any conclusion.

More research in this area is warranted. Several *in vitro* studies report effects of bisphenol A related to carbohydrate and lipid regulation including effects on pancreatic cells that govern the release of insulin (β-cells) and glucagon (α-cells), altered differentiation of fibroblast cells into adipocytes, and altered glucose transport in adipocytes (*206-210*). Some of the effects on pancreatic cells are very rapid, e.g., altered frequency of glucose-induced calcium oscillations in α- and β-cells, activation of cAMP response element binding protein, and appear to be mediated by ncmER (*65, 67, 211*). Effects mediated through the ncmER are of interest because bisphenol A has been shown to activate this receptor *in vitro* at a concentration of 1 nM, which is similar to the active concentration of diethylstilbestrol (*65, 67*).

Decreased Sperm Count and Sperm Quality

There is currently insufficient evidence to conclude that bisphenol A exposure during development or adulthood causes decreased sperm count or sperm quality. A large number of studies have addressed this issue but the literature is inconsistent and not easily reconciled.

- Exposure during development

There are some indications that treatment with "high" oral doses of bisphenol A during development or young adulthood can impact sperm quantity in laboratory rats (*29, 34, 35*). Tan *et al.* (*34*) reported that 33% of rats did not show any evidence of having a spermatogenic cycle after treatment in young adulthood with 100 mg/kg bw/day of bisphenol A. Other reported decreases in measures of testicular or epididymal sperm count and sperm production were more modest and ranged from 10 to 19% at doses of 50 and 500 mg/kg bw/day (*29, 35*). In addition, in

[13] 16 -25 males or females were reported for each treatment group however these animals were derived from only 3 litters per treatment group (i.e., the effective sample size is three instead of 16-25) (*202*).

the three generation rat study conducted by Tyl *et al.* (*29*), significant decreases in sperm parameters were only observed in certain generations of similarly exposed males in the high dose group of 500 mg/kg bw/day: ~18% decrease in epididymal sperm concentration in F1 males; ~19% decrease in testicular daily sperm production in F3 males and no significant effects in the F0 or F2 generations. Testicular or epididymal histopathology was not detected in any treatment group (*29*). Significantly decreased sperm motility and an increased percentage of abnormal sperm has also been reported following "high" dose subcutaneous injection, ~25 mg/kg bw/day[14], to neonatal mice in a study conducted by Aikawa *et al.* (*212*). Again, these effects were not associated with testicular histological alterations.

Effects on sperm parameters have been reported at lower doses administered orally or by subcutaneous injection.[15] vom Saal *et al.* (*213*) reported a ~19% decrease in testicular daily sperm production in adult male mice exposed to bisphenol A as fetuses via maternal dosing with 0.02 mg/kg bw/day (higher dose levels were not tested). Toyama *et al.* (*214*) observed increased incidences of several measures of abnormal sperm morphology (40 – 80% compared to < 0.3% in controls) in mice treated with > 0.17 mg/kg or rats treated with > 0.33 mg/kg by subcutaneous injection[16] of bisphenol A every other day during post-natal days 2 to 12.

However, a number of larger studies have not reported effects on sperm parameters following exposure during development at "high" or "low" dose levels (0.0002 – 600 mg/kg bw/day) (*33, 99, 188-190, 215*).

- Exposure during adulthood only

Several studies have reported effects on sperm parameters in mice or rats exposed to "low" doses of bisphenol A only during adulthood. In rats, these effects are reported following oral dosing of 0.02 – 200 mg/kg bw/day for six days (~24 – 32% decreased daily sperm production per gram tissue) (*216*), 0.0002 – 0.02 mg/kg bw/day for 45 days (~23–41% decrease in epididymal sperm motility; ~18-27% decrease in epidiymal sperm count at 0.002 – 0.02 mg/kg bw/day) (*217*), and 0.0002 – 0.02 mg/kg bw/day for 60 days (~30–45% decrease in epididymal sperm motility; ~12-40% decrease in epidiymal sperm count at 0.002 – 0.02 mg/kg bw/day) (*218*). In adult mice, "low" dose effects on sperm are observed at oral doses of 0.025 – 0.1 mg/kg bw/day for 30 days (~16 – 37% decrease in weight corrected testicular or epididymal sperm count)(*100*) and subcutaneous dosing with 0.02 and 0.2 mg/kg bw/day for 6 days (abnormal sperm morphology) (*219*).

Other larger studies have not reported effects in adult animals at these doses. The 2-generation mouse study conducted by Tyl *et al.* (*33*) reported a 15% decrease in epididymal sperm

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[14] Administered dose was 0.050 mg/pup. This is approximately equal to 25 mg/kg/day assuming that a neonatal mouse weighs 0.002 kg.

[15] Talsness *et al.* (*204*) reported effects on sperm quantity in rats exposed during gestation to 0.1 and 50 mg/kg bw/day but this study is not included in the discussion because (1) reported effects included an increase in sperm number which was opposite the effect observed in the positive control group, and (2) effects on daily sperm production appeared inconsistent over time and across dose.

[16] Administered doses were ≥ 0.001 mg/pup in the mouse and ≥ 0.01 mg/pup in the rat. These doses are approximately equal to 0.17 to 0.5 mg/kg in the mouse and 0.33 - 1.33 mg/kg in the rat assuming that body weight between post-natal days 2 to 12 ranges from 0.002 to 0.006 kg in the mouse and 0.0075 and 0.03 kg in the rat.

concentration in F0 generation animals at the highest dose tested of 600 mg/kg bw/day but not at lower doses of 0.003 to 50 mg/kg bw/day. Ema *et al.* (*99*) also did not detect an effect on sperm measures in the F0 generation in a rat multigeneration study at oral doses of 0.0002 to 0.2 mg/kg bw/day. The finding by Sakaue *et al.* (*216*) of a ~24 – 32% decrease in sperm production in adult Sprague-Dawley rats (obtained from CLEA Japan, Inc.) was not reproduced in a study using larger sample sizes of Sprague-Dawley rats obtained from a Charles River UK (*220*).

The basis for the inconsistent findings is not clear. One proposed explanation is that rodent species, strains, and breeding stocks differ in their responsiveness to estrogens (*51*). Species and strain differences in response to estrogen have been documented, but animal model sensitivity varies depending upon the specific trait being assessed [discussed in (*2, 51, 189*)]. Studies that include sperm assessment in the bisphenol A literature are too varied in terms of periods of dosing, use of positive control, e.g., none used, ethinyl estradiol, or 17β estradiol, and other aspects of experimental conduct to determine if differences in sensitivity of the animal model used can account for the inconsistent findings on sperm quantity and quality.

Chromosome and Meiosis Abnormalities

Disruption of the processes that distribute chromosomes during meiosis or mitosis can result in aneuploid cells, i.e., germ cells that have more or fewer chromosomes than the normal haploid number or somatic cells that have more or fewer chromosomes than the normal diploid number. When this happens in eggs or sperm of humans, it can lead to such conditions as Down Syndrome in which the fetus ends up with 3 copies of chromosome 21, rather than two copies, or a range of syndromes associated with abnormal numbers of sex chromosomes (normal is XX for females, XY for males) such as Klinefelter Syndrome (XXY males) or Turner Syndrome (XO females). If a chemical exposure is capable of inducing aneuploid eggs or sperm, affected individuals would be expected to exhibit problems in achieving or maintaining pregnancy, or to produce aneuploid offspring. While the body of evidence from both *in vitro* and *in vivo* studies provides evidence that bisphenol A can disrupt certain aspects of cell division involving both mitotic and meiotic processes, breeding studies in laboratory animals exposed to bisphenol A do not present results consistent with such effects. Thus, the significance of the reported effects on meiosis and mitosis for mammalian reproduction is not yet clear.

Two *in vivo* studies (*221, 222*) reported that short-term oral exposure to low doses of bisphenol A (≥ 0.020 mg/kg bw/day) in peripubertal or pregnant mice can interfere with meiotic divisions in development of female germ cells ("egg" or "oocyte"). An increase in hyperploid (aneuploid) metaphase II oocytes was observed following treatment with 0.020 mg/kg bw/day. There was not a significant increase in aneuploid embryos. Two subsequent *in vivo* studies (*223, 224*) attempted to replicate these findings. Consistent with the previous findings, they detected no significant effects of bisphenol A exposure on the frequency of aneuploidy in "zygotes" (fertilized oocytes) produced from female mice treated before puberty or as adults with a similar range of doses. In addition, Eichenlaub-Ritter *et al.* (*223*) found no effects of bisphenol A exposure on aneuploid oocytes and Pacchierotti *et al.* (*224*) found no increase in aneuploid or diploid sperm following exposure of male mice to bisphenol A.

A number of *in vitro* studies using cultured mammalian somatic cells have also looked at the potential for bisphenol A to cause aneuploidy. Earlier studies (*225-227*) consistently reported the induction of aneuploidy in various cell lines including SHE, V79, and MCL-5 at concentrations of bisphenol A between 50 and 200 μM (14.4 and 57.6 μg/ml). Recent *in vitro* studies reported effects of bisphenol A on maturation, but not induction of aneuploidy, in mouse oocytes (*223, 228*) or cultured mammalian somatic cells (*229, 230*), increased frequency of mitotic cells with aberrant spindles (*230*), and various effects on cellular and nuclear division in fertilized sea urchin eggs (*231*). Although these new studies provide further evidence of bisphenol A's effects on meiotic and mitotic cell division using a variety of *in vitro* systems and treatment concentrations, no impact of such effects on reproduction is reported in animal breeding studies and the significance of these findings with regard to human health hazards is not clear. If aneuploid eggs or sperm were induced by bisphenol A, it would be expected to result in reduced litter sizes following exposure of one or both parents to bisphenol A. Such an effect is not seen in reproductive toxicity studies of bisphenol A in rats or mice except at very high exposure levels (500 mg/kg bw/day or higher) where other types of toxicities are manifest (*29, 32, 33*), including in the F2 generation (*29, 33*). Findings of significantly decreased litter size or pregnancy loss are reported occasionally at lower doses of bisphenol A (*106, 232*), but in general, most "low" dose studies do not report this outcome including a number of those that report other effects of bisphenol A exposure (*36, 40, 44, 45, 107, 116, 176*).

Are Current Exposures to Bisphenol A High Enough to Cause Concern?

Possibly. The "high" dose effects of bisphenol A in laboratory animals that provide *clear evidence* for adverse effects on development, i.e., reduced survival, birth weight, and growth of offspring early in life, and delayed puberty in female rats and male rats and mice, are observed at levels of exposure that far exceed those encountered by humans. However, estimated exposures in pregnant women and fetuses, infants, and children are similar to levels of bisphenol A associated with several "low" dose laboratory animal findings of effects on the brain and behavior, prostate and mammary gland development, and early onset of puberty in females. When considered together, these findings provide *limited evidence* that bisphenol A has adverse effects on development (**Figure 2b**).

Exposures in humans and laboratory animals can be compared using approaches based on either estimated daily intake (based on aggregating sources of exposure or back calculating from biomonitoring data) or measured blood concentrations of free bisphenol A. Each approach has a unique set of assumptions and limitations. The conclusion of similarities between exposures of certain human populations and laboratory animals treated with "low" doses of bisphenol A is supported by multiple approaches. For this reason, the possibility that human development may be altered by bisphenol A at current exposure levels cannot be dismissed.

Supporting Evidence

A considerable amount of research has been directed towards understanding the levels of human exposure to bisphenol A, either by estimating daily intake or by measuring bisphenol A concentrations in human blood, urine, breast milk, or other tissue. An overarching issue relevant to the bisphenol A biomonitoring studies in both humans and laboratory animals is the accuracy

of the laboratory methods used to measure the compound (see Appendix 1). There is concern that measurements of bisphenol A, especially free bisphenol A, may be too high due to problems related to sample preparation or storage and the analytical technique employed [reviewed in (*2, 11*)]. The NTP recognizes the possibility that the published values of free bisphenol A may, in some cases, not accurately represent the "true" concentrations of free bisphenol A in the blood or body fluids of humans or laboratory animals. However, because of the similarity among values reported with different analytical methods, with the exception of studies that use an enzyme-linked immunosorbent assay (ELISA), the NTP accepts the published values as sufficiently reliable for use in this evaluation.

Daily Intake Exposure Estimates

The vast majority of bisphenol A exposure is through the diet, estimated at ~ 99% (*1*); therefore, estimates of daily intake in humans can be compared to oral doses used in laboratory animal studies where effects considered relevant to human health were observed. Estimates of daily intake are derived using two general approaches. Researchers can use information on the amount of bisphenol A detected in various sources of exposure (i.e., food, food packaging, air, water, dust, etc.) and sum, or aggregate, the measurements to estimate a total daily intake ("aggregating sources of exposure" method). Alternatively, biomonitoring information, such as the concentration of bisphenol A in urine, can be used to estimate, or "back calculate", a total intake that reflects all sources of exposure, both known and unknown. Both approaches for estimating daily intake rely on various assumptions and default values such as average body weight, amount of food or beverage consumed, daily volume of urine output, or ability of a single measurement to characterize exposure.

- Infants and children less than 6 years of age

For infants and children less than 6 years of age, estimates of daily intake were based on aggregating sources of exposure (Table 1). No biomonitoring data, i.e., blood or urine concentration of bisphenol A, are available for these lifestages [reviewed in (*2*)]. An estimated daily intake of ~ 1 µg/kg bw/day for both breast-fed and formula-fed infants was calculated by the CERHR Expert Panel for Bisphenol A (*2*). Higher "worst case" daily intake estimates of 11 - 13 µg/kg bw/day during the first year of life have been calculated for infants (*17*). In children 1.5 to 6 years of age, the range of estimated daily intakes based on aggregating sources of exposure is 0.043 – 14.7 µg/kg bw/day, with 14.7 µg/kg bw/day representing a worst case scenario (*19, 21*).

Although biomonitoring data are not available for infants and children less than 6 years of age, blood and urine levels of free bisphenol A are predicted to be higher in these age groups compared to pregnant women or other adult populations. This is based on information related to age-specific differences in daily intake of bisphenol A and in the ability to metabolize the chemical. More specifically, it is based on observations of (1) higher urinary measurements of total bisphenol A in children (6 - 11 years of age) compared to adolescents and adults (*6*), (2) higher estimated daily intakes of bisphenol A for infants and children (*2, 17, 19*) compared to estimated daily intakes for adults (*2, 17, 27*), and (3) predicted higher blood concentrations of free bisphenol A in infants compared to adults at a given daily intake level based on less efficient

metabolism of bisphenol A in rat fetuses and neonates (*12-14*), and very low or absent activities in human fetuses and premature or full-term infants of the isozymes that govern glucuronidation (*233-235*).

- Adults and children aged 6 years and above

Daily intake estimates for adults and children aged 6 years and older are based on (1) back calculations from the most recent Center for Disease Control and Prevention NHANES data on urinary concentrations of total bisphenol and (2) aggregating sources of exposure (Table 1 and Table 3). Of these estimates, the NTP has more confidence in the estimates based on back calculating from urinary biomonitoring data because all sources of exposure are integrated into the fluid measurement and thus do not have to be identified in advance. However, it is worth noting that the estimates for non-occupationally exposed adults based on aggregating sources of exposure encompass the range estimated from back calculating from urine [aggregating sources of exposure: 0.008 – 1.5 μg/kg bw/day (Table 1); and back calculating based on urine: 0.233 – 0.289 μg/kg bw/day for various categories of adults ages 20+ at the 95th percentile (*27*)]. Fewer studies have estimated daily intakes for children older than 6 years of age and adolescents. In Japanese children and adolescents between the ages of 7 and 19 years, the range of estimated daily intakes based on aggregating sources of exposure is 0.36 to 0.55 μg/kg bw/day (*22*), which is only slightly higher than the estimated range of daily intakes for American children and adolescents based on back calculating from urinary concentration of total bisphenol A [0.311 – 0.348 μg/kg bw/day for children ages 6-11 and 12-19 at the 95th percentile (*27*)].

- Estimated daily intake based on blood biomonitoring

The NTP also considered the appropriateness of estimating daily intake based on back calculations from free bisphenol A measured in human blood and concluded that the scientific uncertainties are currently too large to support this exercise (see Appendix 1). In brief, estimated daily intakes in adults based on this approach are much greater (~500 μg/kg – 1.54 mg/kg bw/day for a 65 kg human) (*3, 236*) than estimates of daily intake based on aggregating routes of exposure (0.008 – 1.5 μg/kg bw/day) (*17, 23*) or from back calculating from urinary data (adults aged 20 – 60+: medians 0.0563 – 0.0334 μg/kg bw/day; 95th percentiles 0.289 – 0.233) (*27*). In addition, data from an intentional dosing study conducted by Tsukioka *et al.* (*237*)[17] provides further support for daily intakes in humans of < 1 μg/kg. Several explanations have been proposed to account for the discrepancy between estimated intake based on blood and urine but they are not sufficient to fully explain it.

Exposure Comparisons Based on Daily Intake

The "high" dose effects of bisphenol A that represent *clear evidence* for adverse effects on development, i.e., reduced survival (≥ 500 mg/kg bw/day) (*28-32*), reduced birth weight and

[17] Tsukioka *et al.* (*237*) used GC/MS with trimethylsilyation (TMS) derivatization (LOQ 0.1 mg/L). Brock *et al.* (*238*) report that use of TMS may produce interfering peaks in the chromatogram. Sample work-up included glucuronidase treatment, solvent extraction, and solid phase clean-up. Few details were presented in the Tsukioka *et al.* (*237*) study on sample preparation process, such as storage temperature.

growth of offspring early in life (≥ 300 mg/kg bw/day) (*28-31, 33*), and delayed puberty in female rats and male rats and mice (≥ 50 mg/kg bw/day) (*29, 33-35*), are observed at dose levels that are more than 3,500-times higher than "worst case" daily intakes of bisphenol A in infants and children less than 6 years of age (≥ 50 mg/kg bw/day versus 0.008 - 0.0147 mg/kg bw/day). The differences in exposures are much greater, more than 160,000-times different, when the high oral dose level is compared to estimated daily intakes for children ages 6-11 and adult women (as an indicator of exposure for pregnant women) at the 95th percentile of 0.311 and 0.271 µg/kg bw/day, respectively (*27*).

However, a number of "low" dose developmental effects have been reported in mice treated orally with bisphenol A including effects on behavior (≥10 µg/kg bw/day) (*36-42*), prostate gland and urinary tract development (10 µg/kg bw/day) (*46*), and early onset of puberty (2.4 and 200 µg/kg bw/day) (*40, 47*). In addition, subcutaneous injection with 10 µg/kg bw/day of bisphenol A during neonatal life in rats results in development of hormonally induced preneoplastic lesions in the prostate later in life (*43*).[18] This non-oral study is considered relevant for comparing exposures because, as discussed previously, the differences in the rate of bisphenol A metabolism seen in adult rats based on route of administration (oral versus non-oral) appear to be greatly reduced in neonatal rats and mice (*12, 58*). As stated earlier, these findings, when considered together, provide *limited evidence* for adverse effects of bisphenol A exposure on development in laboratory animals (**Figure 2b**).

In infants, the doses of 2.4 and 10 µg/kg bw/day are 2.4 - 10 times higher than the estimated daily intake of ~ 1 µg/kg bw/day calculated by the CERHR Expert Panel for Bisphenol A (*2*). Higher "worst case" daily intakes have been calculated for infants by the European Food Safety Authority of 11 - 13 µg/kg during the first year of life (*17*). To the extent these estimates are accurate, then dose levels of 2.4 and 10 µg/kg bw/day slightly exceed (1.1 to 5.4-times) worst case estimates. The doses of 2.4 and 10 µg/kg bw/day are approximately 7.7-32 and 8.9-37 times higher than the estimated daily intakes of 0.311 µg/kg bw/day for children (ages 6-11 years) and 0.271 µg/kg bw/day for adult women at the 95th percentile (*27*).

Exposure Comparisons Based on Blood Concentrations of Free Bisphenol A

No studies in laboratory animals have measured circulating levels of free bisphenol A in the blood following a dosing schedule that mimics human exposures, i.e., long-term dietary low-dose exposure occurring numerous times during the day. However, a number of studies have detected quantifiable levels of free bisphenol A in the blood of adult rodents following a single oral administration of bisphenol A, typically at doses considered high when compared to estimated human daily intakes (500 – 1,000,000 µg/kg for rodents versus < 14.7 µg/kg bw/day for humans) (*3, 19, 27, 236*). These studies were used by Vandenberg *et al.* (*3*) to estimate circulating blood levels of free bisphenol A in rodents at a lower oral dose of 50 µg/kg based on

[18] Preneoplastic lesions in the mammary gland, i.e., ductal hyperplasia and carcinoma *in situ*, have been reported in rats treated as fetuses with 2.5 µg/kg bw/day via a subcutaneous pump implanted in the dam (*44, 45*); however, as discussed previously, studies that administer bisphenol A via subcutaneous pump are considered informative for identifying potential biological effects of bisphenol A, but not for quantitatively comparing exposures in laboratory animals and humans.

the assumption of linear proportionality between administered dose and circulating concentration of free bisphenol A. The estimated peak blood levels of free bisphenol A in the first 30-minutes after dosing at 50 μg/kg ranged from 0.01 to 1.14 μg /L (median 0.11 μg /L) (*3*). Based on this estimate, peak concentrations of free bisphenol A in mice or rats treated with 2.4 or 10 μg/kg bw/day of bisphenol A are projected to be lower than the free blood concentrations measured in humans, including pregnant women (*10, 239*). See Appendix 1 for further details on these calculations.

NTP Conclusions

The NTP concurs with the conclusion of the CERHR Expert Panel on Bisphenol A that there is *some* concern for neural and behavioral effects in fetuses, infants, and children at current human exposures. The NTP also has *some* concern for bisphenol A exposure in these populations based on effects in the prostate gland, mammary gland, and an earlier age for puberty in females.

The scientific evidence that supports a conclusion of *some* concern for exposures in fetuses, infants, and children comes from a number of laboratory animal studies reporting that "low" level exposure to bisphenol A during development can cause changes in behavior and the brain, prostate gland, mammary gland, and the age at which females attain puberty. These studies only provide limited evidence for adverse effects on development and more research is needed to better understand their implications for human health. However, because these effects in animals occur at bisphenol A exposure levels similar to those experienced by humans, the possibility that bisphenol A may alter human development cannot be dismissed.

The NTP has *negligible* concern that exposure of pregnant women to bisphenol A will result in fetal or neonatal mortality, birth defects or reduced birth weight and growth in their offspring.

In laboratory animals, exposure to very high levels of bisphenol A during pregnancy can cause fetal death and reduced birth weight and growth during infancy. These studies provide clear evidence for adverse effects on development, but occur at exposure levels far in excess of those experienced by humans. Two recent human studies have not associated bisphenol A exposure in pregnant women with decreased birth weight or several other measures of birth outcome. Results from several animal studies provide evidence that bisphenol A does not cause birth defects such as cleft palette, skeletal malformations, or grossly abnormal organs.

The NTP concurs with the conclusion of the CERHR Expert Panel on Bisphenol A that there is *negligible* concern that exposure to bisphenol A causes reproductive effects in non-occupationally exposed adults and *minimal* concern for workers exposed to higher levels in occupational settings.

Data from studies in humans are not sufficient to determine if bisphenol A adversely affects reproduction when exposure occurs during adulthood. A number of studies, when considered together, suggest a possible effect on reproductive hormones, especially in men exposed to higher levels of bisphenol A in the workplace. Laboratory studies in adult animals show adverse effects on fertility, estrous cycling, and the testes at exposure levels far in excess of those experienced by humans. A number of other effects, such as decreased sperm counts, are reported for the reproductive system at lower doses in animals exposed only during adulthood, but these effects have not been shown to be reproducible. Laboratory animal studies consistently report that bisphenol A does not affect fertility.

These conclusions are based on information available at the time this brief was prepared. As new information on toxicity and exposure accumulates, it may form the

basis for either lowering or raising the levels of concern expressed in the conclusions.

Figures

Figure 2a. The weight of evidence that bisphenol A causes adverse developmental or reproductive effects in humans.

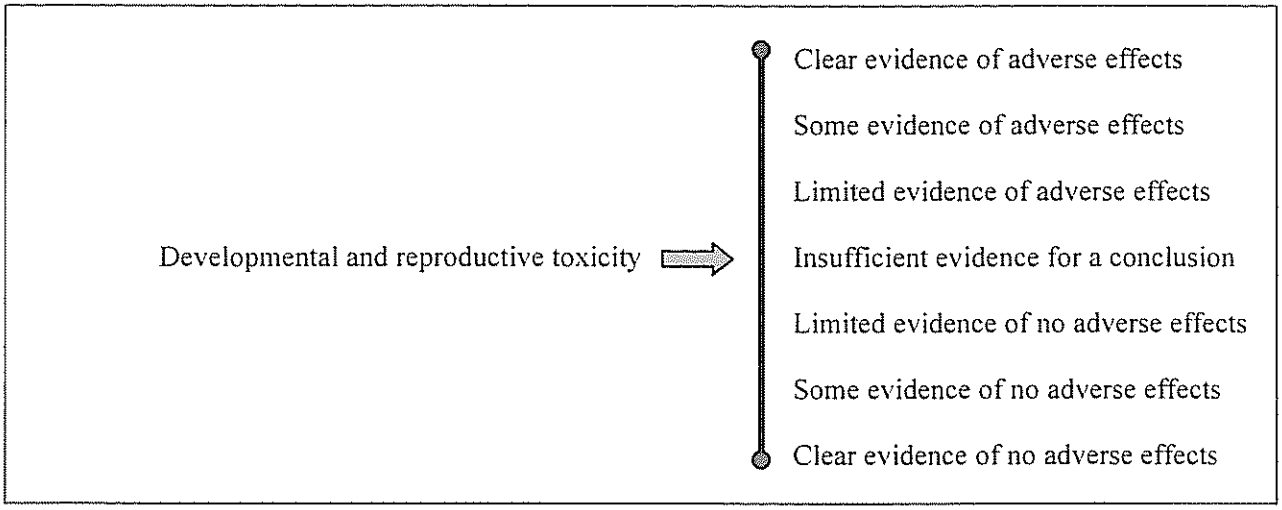
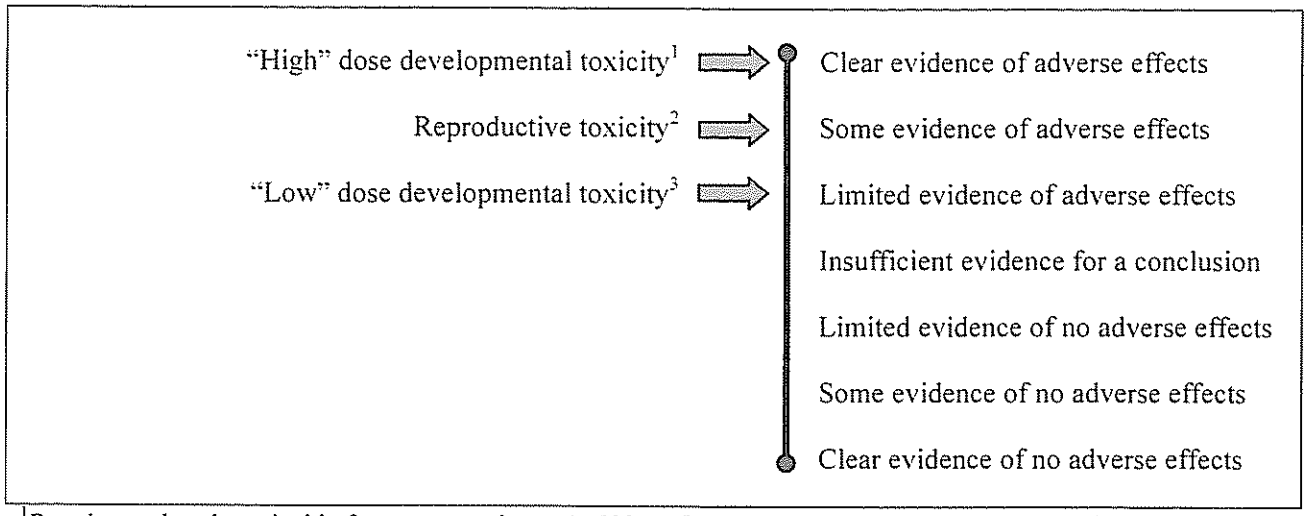


Figure 2b. The weight of evidence that bisphenol A causes adverse developmental or reproductive effects in laboratory animals.

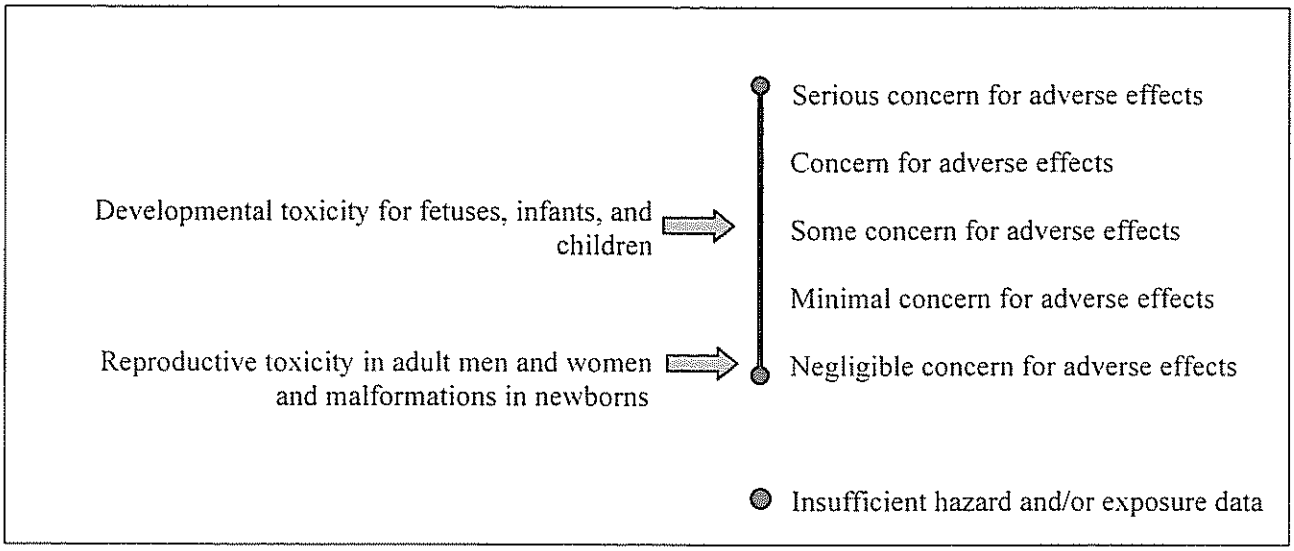


[1]Based on reduced survival in fetuses or newborns (≥ 500 mg/kg bw/day) (*28-32*), reduced fetal or birth weight or growth of offspring early in life (≥ 300 mg/kg bw/day) (*28, 29, 33*), and delayed puberty in female rats (≥ 50 mg/kg bw/day) and male rats and mice (≥ 50 mg/kg bw/day) (*29, 33-35*).

[2]Based on possible decreased fertility in mice (≥ 875 mg/kg bw/day) (*32*); altered estrous cycling in female rats (≥ 600 mg/kg bw/day) (*102*), and cellular effects on the testis of male rats (235 mg/kg bw/day) (*103*).

[3]Based a variety of effects related to neural and behavior alterations (≥10 µg/kg bw/day) (*36-42*), precancerous lesions in the prostate (10 µg/kg bw/day) (*43*) and mammary glands (0.0025 – 1 mg/kg bw/day) (*44, 45*); altered prostate gland and urinary tract development (10 µg/kg bw/day) (*46*), and early onset of puberty (2.4 and 200 µg/kg bw/day) (*40, 47*).

Figure 3. NTP conclusions regarding the possibilities that human development or reproduction might be adversely affected by exposure to bisphenol A



Appendix 1: Interpretation of Blood Biomonitoring Studies

Free bisphenol A has been measured in the blood of pregnant women at concentrations up to 22.4 µg/L (*10*). How to account for the detection of free bisphenol A in human blood is an area of scientific debate. In a controlled and intentional dosing study in humans, free bisphenol A was not detected in the blood or urine of a small number of adult subjects (n=9) orally dosed with 5 mg/person bisphenol A, ~54 – 90 µg/kg (*240*). This dose range is approximately 200 to 400-fold higher than the estimates of daily intake based on urinary biomonitoring data for adults (95th percentile of 0.233 – 0.289 µg/kg bw/day) (*27*). The findings by Völkel *et al.* (*240*) lead to the prediction that the capacity for conjugation reactions is so large in humans that free bisphenol A should not be present in detectable concentrations in the blood of non-occupationally exposed adults. However, biomonitoring studies of the general population report detecting free bisphenol A in the blood, including from pregnant women (*10, 239*), urine (*241*), and breast milk (*5*). Despite the relatively high limit of detection of the analysis method for free bisphenol A of 2.28 µg/L (10 nM) for blood in the 2002 study by Völkel *et al.* (*240*), it is a source of scientific uncertainty why free bisphenol A was not detected in this study in light of reports of mean blood concentrations of free bisphenol A up to 4.4 µg/L (*239*) and 5.9 µg/L (*10*) in pregnant women in the general population.

This discrepancy has contributed to the concern expressed by some scientists that the reported detections of free bisphenol A are artifacts of problems related to sample preparation or storage and the analytical technique employed (*2, 11*). Ideally, methods should measure only bisphenol A and not other compounds ("specificity"). There is scientific consensus that measurements of bisphenol A based on the enzyme-linked immunosorbent assay (ELISA) are the least reliable and non-specific due to potential cross-reactivity with structurally-similar compounds (*2, 3, 11*)[19]. Analytical methods should also be able to detect bisphenol A at low concentrations ("sensitivity"). In addition, measurements of free bisphenol A should be based on analytical methods that accurately distinguish between the concentrations of free bisphenol A and its conjugated metabolites.

There is concern that current measurements of free bisphenol A may be too high (*2, 11*). This could occur, for example, if the method used misidentified other chemicals as bisphenol A or if there was background contamination from laboratory ware. Alternatively, the procedures used to process the samples could introduce bias in measurement even if the analytical method employed is high quality. Measurements of free bisphenol A could be overestimated if the samples were processed in a manner that allowed the conjugated metabolites to revert back to the free form of bisphenol A. For example, conjugated bisphenol A in urine only appears to be stable when stored at room temperature for ~24 hours. After 2 – 4 days at this temperature conjugated bisphenol A begins to degrade and the percent detected in samples decreases ~ 8 to 30%, i.e., higher concentrations of free bisphenol A would be detected over time (*242*).

However, free bisphenol A has been detected in 10% of human urine samples [range = < limit of detection (0.3) – 0.6 µg/L; n = 30] (*241*) and in 60% of breast milk samples [mean = 1.3 µg/L;

[19] Analytical techniques used to measure bisphenol A include gas chromatography/mass spectrometry (GC/MS), liquid chromatography/mass spectrometry (LC/MS), high performance liquid chromatography (HPLC) with fluorescence or electrochemical detection, and enzyme-linked immunosorbent assay (ELISA).

median = 0.4 μg/L; range = < limit of detection (0.3) – 6.3 μg/L; n = 20] (*5*) by researchers at the CDC who use analytical methods considered by many scientists to be very accurate (the CDC has not presented data on measurements of bisphenol A in blood). A proposed explanation to account for the detection of free bisphenol A in breast milk is that the free form of bisphenol A is more lipophilic than the conjugated forms and therefore more likely to sequester in breast milk (*5*).

In addition, Tsukioka *et al.* (*237*) were able to detect free bisphenol A in the urine of all human subjects treated with ~ 0.83 μg/kg, whereas Völkel *et al.* (*240*) was unable to detect any free bisphenol A in subjects treated with doses 65 – 108-times higher, ~54 – 90 μg/kg. It cannot be definitively determined if the detection of free bisphenol A in urine in the study by Tsukioka *et al.* (*237*) was due to the analytical method employed or partial cleavage of glucuronide during sample storage, preparation or analysis. However, Tsukioka *et al.* (*237*) also detected total and free bisphenol A in the urine of subjects that were not intentionally treated [total bisphenol A: 0.82 μg/L (range 0.14 - 5.47; n = 91); free bisphenol A: 0.08 μg/L (range 0.01 - 0.27 ng/m; n = 11)] and these values are lower than CDC measurements of total [2.6 μg/L for all subjects in the NHANES study (*6*)] and free bisphenol A [10 of 30 subjects at <LOD(0.3) - 0.6 μg/L (*241*).

In summary, the NTP recognizes the possibility that the published values of free bisphenol A may, in some cases, not accurately represent the "true" concentrations of free bisphenol A in the blood or body fluids of humans or laboratory animals. However, because of the similarity among values reported with different analytical methods, with the exception of ELISA-based studies, the NTP accepts the published values as sufficiently reliable for use in this evaluation.

Comparison of measured human blood concentrations of free bisphenol A with estimated concentrations in laboratory rodents at low doses

More than ten toxicokinetic and metabolism studies have detected quantifiable levels of free bisphenol A in the blood of adult rodents, mostly rats, following oral administration of doses that are considered high when compared to estimated human daily intakes (500 – 1,000,000 μg/kg for rodents versus < 14.7 μg/kg bw/day for humans) (*3, 19, 27*) (Table 1 and Table 2). These studies were used by Vandenberg *et al.* (*3*) to estimate circulating blood levels of free bisphenol A in laboratory rodents at a lower oral dose of 50 μg/kg bw/day based on the assumption of linear proportionality between administered dose and circulating concentration of free bisphenol A. The estimated peak blood levels of free bisphenol A achieved in the first 30 minutes after dosing ranged from 0.01 to 1.14 μg/L (*3*).

Using the estimates provided by Vandenberg *et al.*(*3*) for peak blood levels of free bisphenol A at 50 μg/kg and again relying on the assumption of linear proportionality, the NTP estimated the range of peak concentrations of free bisphenol A at 10 μg/kg, a dose where a number of "low" dose effects are reported, to be five times lower, i.e., 0.002 to 0.228 μg/L. These values are 2950 to 25.9 times lower than the mean blood concentration of free bisphenol A detected in pregnant women in Michigan (5.9 ± 0.94 μg/L; range 0.5 to 22.4) (*10*).

The appropriateness of extrapolating from higher dose studies to predict blood levels of free bisphenol A at lower dose levels rests on the validity of the assumption of proportionality. This assumption is warranted if, for example, blood levels of free bisphenol A are approximately 10 times lower following dosing with 10 mg/kg than after dosing with 100 mg/kg. Three studies are

available that used non-ELISA methods to measure concentrations of free bisphenol A following oral dosing with 10 and 100 mg/kg bisphenol A in adult rats (*59, 243, 244*). In these studies, the peak, or C_{max}, blood concentrations of free bisphenol A were 4.8-times (*243*), 22.7-times (*244*), and 57-times (*59*) lower in rats treated with a 10 mg/kg dose compared to rats treated with 100 mg/kg.

Directly evaluating proportionality at lower oral doses (< 10 mg/kg) has not been possible in adult animals because blood concentrations of free bisphenol A are below the limits of detection for the analytical methods employed. One strategy that can be used to address the assumption of proportionality at low doses is to rely on studies that have dosed young rodents because they have higher peak blood concentrations of free bisphenol A compared to adults treated with the same dose (*12*). Two studies have measured concentrations of free bisphenol A in young rodents at more than one dose level (*12, 58*). In 3-day old female mice orally treated with 0.035 and 0.395 mg/kg bisphenol A, Taylor *et al.* (*58*) found that the peak blood concentration of free bisphenol A at 0.035 mg/kg was 8.3-times lower than the peak concentration at 0.395 mg/kg (difference between administered does is 11.3-times). The study by Domoradzki *et al.* (*12*) treated neonatal rats orally with higher doses of bisphenol A than those used by Taylor *et al.* (*58*). In 4-day old female and male rats, the peak concentrations of free bisphenol A were 170 to 1610-times lower at 1 mg/kg compared to 10 mg/kg bisphenol A. This finding, coupled with data for 21-day old rats presented in Domoradzki *et al.* (*12*) and the comparisons presented above from Tominaga *et al.* (*244*), and Pottenger *et al.* (*59*), suggest that rodents, and presumably humans, can more efficiently metabolize lower doses of bisphenol A compared to high doses. These data also suggest that extrapolating from higher dose levels in the mg/kg range may overestimate the circulating concentrations of free bisphenol A following administration of oral doses in the low µg/kg range.

Any extrapolation and use of assumptions involves some degree of uncertainty. However, the conclusion outlined above of similar blood levels in the general population and in laboratory animals at "low" doses would still hold even if the estimated blood levels of free bisphenol A in laboratory rodents were overestimated by a factor of 100 or 1000, i.e., the "real" peak blood values in laboratory animals range from 0.2 to 22.8 or 2 to 228 µg/L instead of the estimated 0.002 to 0.228 µg/L.

This possibility that blood concentrations of free bisphenol A in humans could be significantly higher, as much as ~3000 times greater, than the estimated peak concentrations in laboratory animals where biological changes are observed is a point of intense scientific controversy. In brief, although the theoretical plausibility of receptor-mediated effects at "low" doses has been described (*245, 246*), many scientists expect that a compound with a significant degree of biological "activity" at low doses would show more profound impacts on overall toxicity at lower doses than that observed for bisphenol A. With bisphenol A, "low" dose developmental effects can be observed at 0.0024 to 0.010 mg/kg bw/day but indications of severe developmental toxicity in rats and mice, i.e., fetal or neonatal death are not observed except when doses are used that are 50,000 – 200,000-times higher at ≥ 500 mg/kg bw/day (*28-32*).

Estimated daily intake based on back calculating from blood and urine

Based on parameters derived from laboratory animal studies, estimated daily intakes based on back calculations from free bisphenol A measured in human blood are much greater (~500 µg/kg –

1.54 mg/kg bw/day for a 65 kg human) (*3, 236*) than estimates based on any other approach. In contrast, there is a degree of concordance in estimates of daily intake based on other approaches. For these reasons, the NTP has less confidence in daily intake estimates based on blood biomonitoring data compared to other estimates, particularly those based on urine biomonitoring data.

Estimates of daily BPA intake in adults based on aggregating routes of exposure fall within the range of 0.008 – 1.5 μg/kg bw/day (*17, 23*) (Table 1) with most estimates falling within a range that spans one order of magnitude, 0.183 – 1.5 μg/kg bw/day (*16-19, 22*). Daily intakes estimated from the CDC NHANES biomonitoring data are similar and range from 0.289 – 0.233 μg/kg bw/day for adults aged 20 – 60+ years at the 95th percentile (*27*). The NTP considered the possibility that the assumptions used to derive these intakes could underestimate human exposures. For estimates based on aggregating sources of exposure, one concern is that too much emphasis has been placed on diet as the predominant route of exposure. For estimates based on the total concentration of bisphenol A in urine, it is assumed that the daily excretion of bisphenol A is a reasonable surrogate for daily intake. Deviations from the assumptions used to derive current estimates could increase the daily intake estimates, but still result in estimated intakes in the very low μg/kg bw/day range rather than near 1 mg/kg bw/day as predicted from the blood biomonitoring data in adult humans.

Data from an intentional dosing study conducted by Tsukioka *et al.* (*237*) provides further support for daily intakes of < 1 μg/kg. Tsukioka *et al.* gave 15 volunteers (12 men and 13 women) 50 μg of bisphenol A by mouth (~ 0.83 μg/kg for a 65 kg person) and collected urine samples for 5 hours. The average concentration of total bisphenol A was 57.2 μg/L (range 26.5 - 80 μg/L) and free bisphenol A was 1.13 μg/L (range 0.13 - 5.8 μg/L). The administered dose, ~0.83 μg/kg, and urinary concentration of total bisphenol A, 57.2 μg/L, are ~14.8-times and 18.5-times higher, than the estimated median intake of 0.056 μg/kg bw/day for adults aged 20-39 years based on a median urinary concentration of 3.1 μg/L calculated by Lakind *et al.* (*27*). Extrapolating downward for administered dose and urinary concentrations of total bisphenol A from the data provided by Tsukioka *et al.* (*237*) would give values that are consistent with the daily intake calculated by Lakind *et al.* (*27*) based on the CDC urinary measurements (*6*).

Exposure Assessment Research Needs

The NTP concurs with the CERHR Expert Panel on Bisphenol A that more measurements in humans are needed of free and total bisphenol A, its glucuronide conjugate, and other metabolite concentrations from maternal, fetal, and neonatal tissues or fluids (i.e., placenta, amniotic fluid, breast milk, urine, serum). These data would provide further insight into the roles of metabolism and exposure route on internal dose and provide a firmer foundation for extrapolations of risks to humans from the wealth of animal studies available. Available data demonstrate that a large fraction of children and adults have detectable levels of bisphenol A, or it's metabolites, in their urine. Duplicate diet studies to identify in detail the sources and routes of exposure of bisphenol A would be useful. For example, while research suggests diet is the major source of bisphenol A for infants and young children in the United States, the detailed analysis of bisphenol A levels has primarily focused on polycarbonate baby bottle leachates and canned food. The contributions of non-canned food and drinking water routes of exposure for youth and adults not occupationally-exposed to BPA remain unknown and in need of further study. Levels of bisphenol A in

residential drinking water wells and community water sources have not been systematically studied. Also unknown is the impact of landfill leachates on levels of bisphenol A in U.S. drinking well waters and whether chlorinated congeners of bisphenol A are found in municipal water supplies.

More research is needed to characterize the toxicokinetics of bisphenol A in developing animals under exposure scenarios that better mimic the low-level chronic exposures experienced by humans. Currently, only single or "acute" dosing kinetic studies in laboratory animals are available for predicting the metabolism and fate of bisphenol A following long-term, daily exposure, or for comparing apparent differences in the metabolism and fate of bisphenol A in laboratory rodents and humans. Repeated administration of many compounds has been shown to alter the capacity of the animal to metabolize and excrete the compound. Further characterization of the ability of repeated exposures to bisphenol A to change rates and extents of metabolism and excretion in laboratory animals and humans is a critical research need.

In addition, it is clear that there are differences in the pharmacokinetics of bisphenol A, particularly between rats and humans that complicate using the rat data to interpret the human biomonitoring data. For example, the excretion profiles of bisphenol A differ in rodents and humans. In humans, the major route of elimination is via the urine in the form of bisphenol A glucuronide (*247*). In contrast, the major elimination routes in rodents are as bisphenol A in the feces and as bisphenol A glucuronide in the bile and, to a lesser extent, in the urine [reviewed in (*2*)]. Also, in rats bisphenol A glucuronide can remain in the bile and be re-circulated back to the liver ("enterohepatic circulation"). Development of physiologically-based pharmacokinetic (PBPK) models is needed to facilitate the interpretation and applicability of animal studies for human risk assessment.

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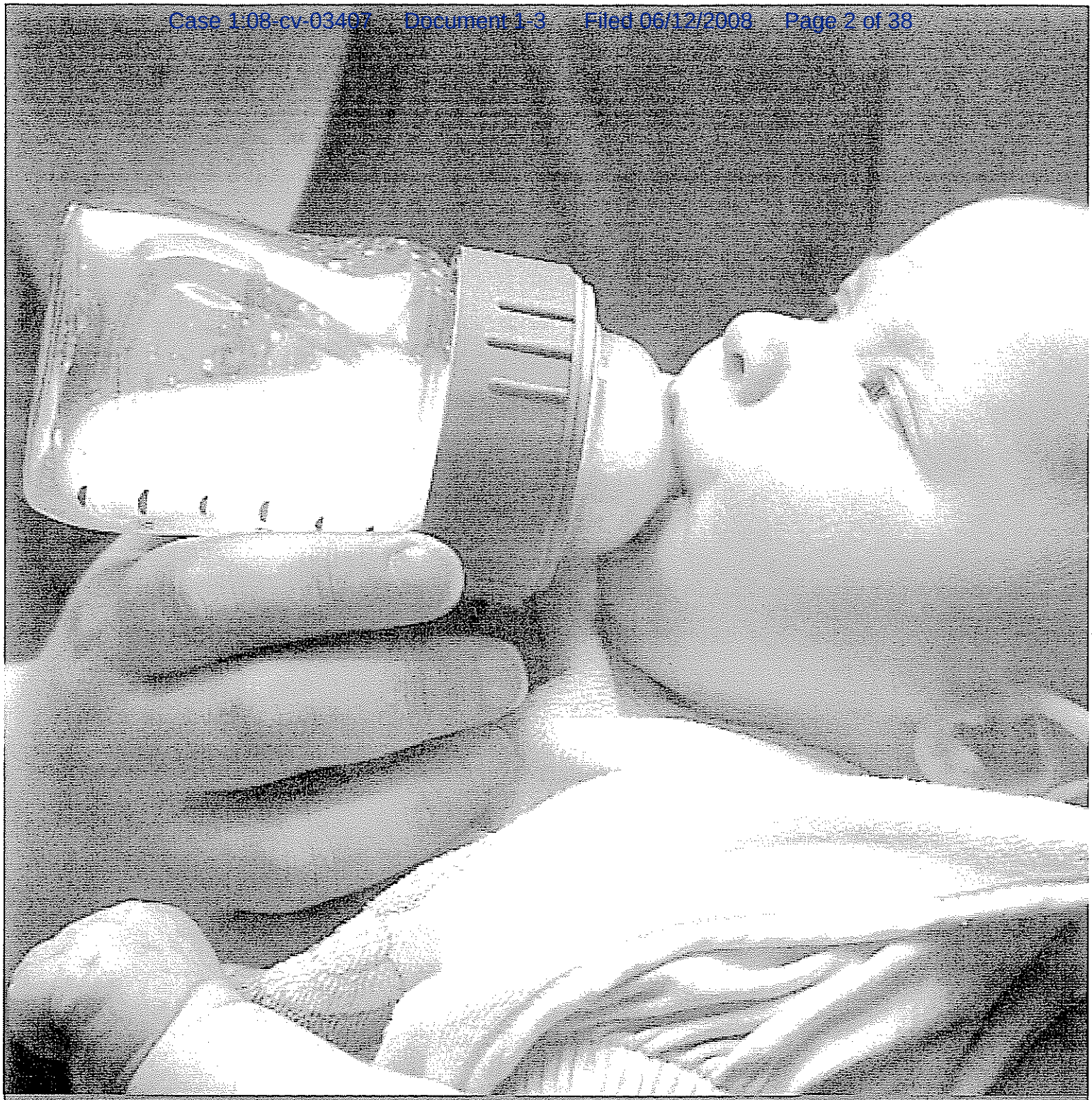
Exhibit B



Toxic Baby Bottles

Scientific study finds leaching chemicals in clear plastic baby bottles

ENVIRONMENT CALIFORNIA

2007

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Written by
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Environment California Research and Policy Center

2007

ACKNOWLEDGMENTS

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Environment California Research & Policy Center would like to thank those who provided technical and editorial support, guidance, or review, including Dr. Fred vom Saal and Dr. Wade Welshons of the University of Missouri-Columbia; Travis Madsen of Frontier Group; Gretchen Lee of Breast Cancer Fund; Joe Guth of Science and Environmental Health Network; and Nikki Riedt of Environment California Research & Policy Center. Environment California Research & Policy Center also would like to thank Mary Brune of Making Our Milk Safe (MOMS) for her thoughtful introduction to the report. Thanks also to Kathleen Krushas and To the Point Publications for layout design, Justin Boyles for cover design, and Shutterstock for the cover photo.

Environment California Research & Policy Center is grateful to the California Wellness Foundation, the Clarence E. Heller Charitable Foundation, the Fred Gellert Family Foundation, and the individual contributors who helped make this report possible.

The author alone is responsible for any factual errors. The views expressed in this report are those of the author and do not necessarily reflect the views of our funders, those who provided editorial review, or their employers.

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Executive Summary

Products marketed for infants and children are not always completely safe for their use. Many contain toxic chemicals that may have detrimental health impacts for children exposed during critical stages of development.

In this report, we analyze the extent to which five popular brands of baby bottles leach bisphenol A, a developmental, neural, and reproductive toxicant, into liquids coming into contact with them. We found that all five brands leach bisphenol A at dangerous levels found to cause harm in numerous laboratory animal studies.

California and the U.S. should reform chemical policy to ensure that all products on the market are safe for children.

Bisphenol A is a Developmental, Neural, and Reproductive Toxicant

- Scientists have linked very low doses of bisphenol A exposure to cancers, impaired immune function, early onset of puberty, obesity, diabetes, and hyperactivity, among other problems.
- For example, in one recent study, a single, low dose of bisphenol A administered to a newborn rat resulted in hyperactive behavior.

Exposure to Bisphenol A is Widespread

- Bisphenol A is most commonly used to make clear polycarbonate plastic for consumer products, such as baby bottles. Through use, this plastic breaks down and leaches bisphenol A into liquids and food to which it comes into contact.
- The U.S. Centers for Disease Control and Prevention found bisphenol A in the urine of over 95% of people they tested.
- Alarmingly, the median level of bisphenol A in humans is higher than the level that causes adverse effects in animal studies.

Popular Baby Bottles Sold in California Leach Bisphenol A at Harmful Levels

- Based on a consumer survey of the most popular baby bottle brands on the market, we selected five bottle types to determine the amount of leaching from each bottle. We found that the bottles tested from all five brands leached bisphenol A at levels found to cause harm in numerous laboratory studies, including:
  - Avent
  - Dr. Brown's
  - Evenflo
  - Gerber
  - Playtex

Recommendations for Parents

Parents have the right to know about chemicals in the products they purchase for their children. In the absence of good government regulations, but armed with the knowledge that some chemicals are a cause for concern, parents can take a few simple actions to limit their child's exposure to these and other toxic chemicals.

At the store, parents should select baby bottles that are made from glass or a safer non-polycarbonate plastic. At home, parents should avoid washing plastic dishware with harsh dishwashing soap and hot water, which may allow chemicals to leach out of the plastic. For a useful tip sheet, parents should visit www.EnvironmentCalifornia.org.

Recommendations for Policymakers

Parents cannot deal with these issues alone. The government must ensure the safety of all products on the market for children. California and the U.S. should:

Phase Out Hazardous Chemicals

Based on the weight of the scientific evidence showing the harm caused by exposure to bisphenol A, the government should act now. Given that data from the U.S. Centers for Disease Control and Prevention show that bisphenol A is present in humans at levels found to be harmful in laboratory studies, California and the U.S. should phase out the use of bisphenol A, especially in products used by children.

Inform Consumers about the Presence of Dangerous Chemicals

Parents currently have little information to inform their decisions when purchasing products for their family. Manufacturers should be required to label children's products with the name of any potentially dangerous chemical and the specific health risks associated with the chemical.

Reform Chemicals Policy

Currently, manufacturers can put chemicals on the market without proving they are safe. Chemical manufacturers should be required to provide all hazard and health-effects information to the government so agencies can begin to assess the thousands of chemicals currently on the market for which little or inadequate data are available. Next, pre-market hazard and health-effects testing should be required for all new chemicals before they are introduced into commerce. Finally, the California Environmental Protection Agency must have the authority to protect public health by banning or restricting the use of a chemical if evidence shows that it can harm human health.

INTRODUCTION

Becoming a mother was an amazing and alarming experience for me. Amazing because my daughter was *finally* here. Alarming because now that she was here, there was suddenly so much to worry about. Is she nursing enough? Sleeping enough? Sleeping too much? I learned early on that the questioning never really ends. The difficulty lies in knowing which questions to ask.

After I returned to work, my daughter began attending daycare. I continued to feed her breast milk, which had been stored and frozen in plastic baby bottles. At the time, it felt good to know that she would be getting all the benefits of breast milk even while I was away. What I didn't realize at the time, was that in addition to the enzymes and antibodies she would receive as she drank from the bottle, she also could be exposed to a chemical linked to dangerous developmental and reproductive health effects.

There is a wealth of information available to help new parents choose the safest product at the best value for their babies. Whether it's safety ratings on that new car seat, or a friend's recommendation on the sturdiest stroller, parents have the information they need to make informed choices about most of the products they buy for their child. Except, that is, when it comes to determining which products might contain dangerous chemicals.

Toxic chemicals, many linked to significant health problems, can be found in a wide variety of children's products. Sadly, baby bottles are no exception. Most popular baby bottles on store shelves are made with bisphenol A, a chemical known to disrupt the hormone system even when exposed to extremely low doses. And like me, most parents have no idea that a dangerous chemical is lurking in their baby's bottle.

Even the most educated parents have a hard time figuring out which products are safe and which may be harmful. I've spent hours researching everything from the safest bottle to the safest teether, and yet, it's impossible to know with any certainty whether an individual product falls into the safe or hazardous category.

The only way children will be protected from dangerous chemicals is for the government to take bold steps and prohibit the use of chemicals in children's products that are known or suspected of causing harm.

Parents must speak out. I was scared when I first started uncovering information about the dangerous chemicals found in common children's products. But I turned the fear into action. Parents can and should reach out to their elected officials to demand that they do more to protect children's health.

Mary Brune
Co-founder, Making Our Milk Safe (MOMS)

Bisphenol A: Developmental, Neural, And Reproductive Toxicant

Bisphenol A is a chemical commonly used in the manufacture of clear polycarbonate plastic. It is one of the top 50 products produced by the chemical industry, generating revenues on the order of $6 million per day in the United States, Europe, and Japan alone.[1] Global bisphenol A production exceeds 6.4 billion pounds per year.[2]

Bisphenol A can be found in a wide variety of consumer products, including clear plastic baby bottles. Dozens of other common household and consumer items contain bisphenol A, including some types of reusable water bottles and microwaveable food containers, electronic equipment, automobiles, sports helmets and pads, eyeglass lenses, and more. Bisphenol A is also used in epoxy resins found in white dental sealants, printed circuit boards, paints, glues, protective coatings, and—more worrisome—in the lining of metal cans containing food and drink.[3] It is also an additive in other types of plastic used to make children's toys.

However, bisphenol A is also a developmental, neural, and reproductive toxicant. When in the body, it can act as a substitute for the female hormone estrogen, interfering with the normal process of signaling that is critical for the healthy growth, development, and function of the human body.

Scientists first learned that bisphenol A could act as a synthetic substitute for estrogen in the 1930s, close to 30 years after its invention.[4] It wasn't until 1953 that chemists discovered bisphenol A could be made into polycarbonate plastic. Despite the fact that bisphenol A was known to mimic estrogen, it went on to become commonplace in the manufacture of a variety of consumer products.

A Small Sample of Bisphenol A Uses Include...

...polycarbonate plastic, including most plastic baby bottles
...children's toys
...dental sealants
...epoxy lining of food and beverage cans
...reusable drink containers
...microwavable food containers
...electronic equipment
...sports helmets
...eyeglass lenses

Bisphenol A Causes Health Problems

Extensive scientific literature reports adverse health effects from bisphenol A at very low doses. Studies show that bisphenol A can alter the expression of several hundred genes with effects varying among specific tissues and depending upon the timing of exposure. More than 150 laboratory animal studies suggest that bisphenol A exposure at very low doses is linked to a staggering number of health problems, including prostate and breast cancer, obesity, hyperactivity, diabetes, altered immune system, lowered sperm count, and early puberty.

Adverse Health Effects of Bisphenol A Include...

...early onset of puberty
...obesity
...diabetes
...hyperactivity
...increase in aggression
...changes in response to painful or fear-provoking stimuli
...impaired learning and memory
...reversal of normal sex differences in the brain structure
...elimination of sex differences in behavior
...decreased maternal behavior
...impaired immune function
...breast cancer
...prostate disease and cancer
...sperm defects
...impaired female reproductive development
...miscarriage

Children are Most at Risk

Growing children are particularly at risk to toxic chemicals in their environment because they are physiologically more susceptible to them.[5] Children's exposures begin at conception, as chemicals, including bisphenol A, cross the placenta in a pregnant woman's body, potentially affecting the embryo or fetus during critical periods of development.[6] Even after birth, children's bodies remain immature with underdeveloped detoxification mechanisms to protect them from toxic chemicals. Children's brains and other organ systems are constantly developing, undergoing periods of particular sensitivity to damage or disruption.

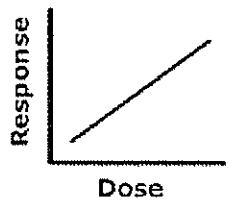
Like over-the-counter medications, which children's bodies cannot tolerate or can only tolerate in extremely low levels, children are particularly susceptible to the harmful effects of bisphenol A. However, there is now extensive evidence that many of the problems associated with bisphenol A exposure during these critical stages of development may not come to light until years after exposure.

Especially because growing children are particularly at risk from bisphenol A exposure and because adverse effects on intellectual ability, social behaviors, fertility, and potential for disease may take decades to detect, measures must be taken to protect children from exposure to products containing bisphenol A that they use every day.

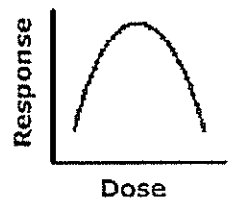
The Dose Does Not Make the Poison

For decades, scientists in the field of toxicology have assumed that the higher the dose of a chemical the greater the harm. Decades of studies of hormones by endocrinologists, and recent application of methods used to study hormones to the study of hormone-mimicking chemicals such as bisphenol A, invalidate this prediction that the dose makes the poison. Numerous studies show that bisphenol A and other hormone-mimicking chemicals result in great harm at very low doses that is not predicted by studies with only very high doses. Rather than having a linear dose-response curve, the dose-response curve for bisphenol A appears more like an inverted "U" in which lower doses of exposure cause greater harm than higher doses. The standard tests used in toxicology to set health standards have assumed that the dose makes the poison, thereby ignoring the low-dose impacts of chemicals that mimic hormones. The implications of this fact are stark: the health standards set by the government may not in reality be protecting human health.

Linear Response Curve



Inverted U-Shaped Curve



Bisphenol A Can Induce Chromosome Sorting Errors

Bisphenol A recently burst onto the scene as a potential factor in the incorrect sorting of chromosomes. In 2003, Dr. Pat Hunt and her colleagues made an accidental but dramatic discovery: bisphenol A can cause chromosomes to sort incorrectly, even at very low doses.[7] Germ cells normally split into two cells when forming eggs, separating chromosomes equally into each daughter cell. These cells then enter the reproductive process, and when fertilized by sperm, develop into new organisms. Dr. Hunt showed that exposure to bisphenol A prevents the chromosomes from lining up correctly, resulting in chromosome sorting errors like the kind that cause Down syndrome. While a variety of possible events could also lead to the same genetic outcome, the fact that a common chemical can cause this effect is cause for concern.

The Bisphenol A – Down Syndrome Connection

When chromosomes sort incorrectly in a father's sperm or mother's egg, diseases—and, quite frequently, miscarriages—result. Incorrect sorting of chromosomes leads to diseases like Down syndrome, in which a child has an extra copy of chromosome 21 and suffers multiple mental and physical impairments; Turner syndrome, in which a female has only one X-chromosome and never develops ovaries; and Klinefelter syndrome, in which a male has one or more extra X-chromosomes and is sterile. Dr. Hunt's findings show that extremely low doses of bisphenol A exposure are linked to an error in cell division called aneuploidy, which causes 10-20 percent of all birth defects in people, including Down syndrome.[8]

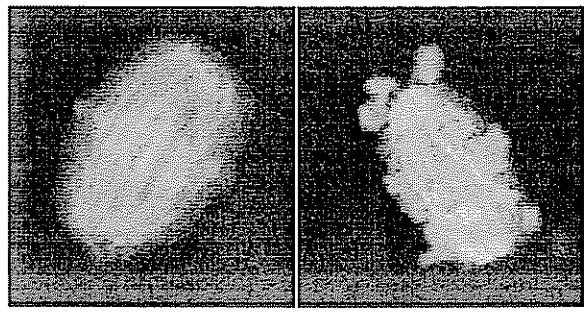
While a variety of possible events could also lead to the same genetic outcome, the fact that a common chemical can cause this effect is cause for concern.

At the time of Dr. Hunt's discovery linking bisphenol A to chromosome sorting errors, her research team was not studying bisphenol A. The lab was using mice for their research, and lab staff kept the mice in plastic cages and fed them water from plastic water bottles. The staff were shocked when they discovered severe chromosome sorting problems in developing egg cells of mice they were expecting to be normal. Dr. Hunt faced the question of how untreated mice developed such striking damage to their egg cells.

The answer to the mystery turned out to be contamination from the polycarbonate plastic cages and water bottles. Bisphenol A leached out of these items into the mice in appreciable quantities. Lab staff were able to replicate the effect in several ways: by feeding mice through polycarbonate plastic bottles purposefully washed to accelerate leaching of bisphenol A, and by directly administering small doses of pure bisphenol A to the mice.

Even at the lowest dose tested of 20 micrograms per kilogram per day (20 µg/kg/day) for 6 to 8 days, Dr. Hunt found that bisphenol A caused significant and observable damage to developing eggs (Figure 1, next page).

Figure 1: Bisphenol A Causes Chromosomes to Sort Incorrectly During the Development of Egg Cells[9]



In normal development (left), eggs and sperm develop when a germ cell splits in two, giving an equal set of chromosomes to each germ cell. The chromosomes (red) line up on the spindle (green) to ensure equal separation. However, bisphenol A prevents the chromosomes from lining up correctly (right), resulting in chromosome sorting errors like the kind that cause Down syndrome.

Interviewed by the *Los Angeles Times* about this finding, Dr. Frederick vom Saal at the University of Missouri, a leading bisphenol A scientist, noted that "these effects in the Hunt study and other studies happen at lower doses than what is actually found in human fetal blood—umbilical cord blood." In fact, tests of placental tissue and amniotic fluid of women in Germany and Japan found bisphenol A at high levels—from 1 to 105 parts per billion (ppb), which is above the range found in mice administered doses that caused chromosome sorting errors in Dr. Hunt's study.[10]

Subsequent research by Dr. Hunt and her colleagues shows that exposure to bisphenol A can lead to chromosomal abnormalities that affect future generations as well.[11] This is because female mammals, including mice and humans, form their eggs while still in their mother's womb. Thus, the eggs that will become a female's grandchildren are affected through in utero exposure to bisphenol A.

Prior to this finding by Dr. Hunt and her colleagues, researchers believed that fetal exposure to bisphenol A could be avoided simply by staying away from the chemical during pregnancy. Dr. Hunt's research team demonstrated, however, that "[bisphenol A] can lie inside [a female] like a time bomb ready to detonate once she becomes pregnant."[12] This is a classic example of the consequences of fetal exposure not being realized until long after the exposure occurred.

Specifically, in this recently published study, Dr. Hunt exposed pregnant female mice to low doses of bisphenol A in the 20 µg/kg/day range. Dr. Hunt found that the undeveloped eggs inside the developing fetuses of the exposed mice showed chromosomal abnormalities. As many as 40 percent of these eggs had chromosome abnormalities. Normally less than 1 percent would show problems. The abnormalities suggest that the eggs would not be able to create viable offspring, highlighting the concern that bisphenol A could affect the grandchildren of an exposed pregnant female.

Bisphenol A Can Lead to Early Onset of Puberty

Bisphenol A from polycarbonate plastic accelerates the timing of puberty in laboratory studies. Indeed, several studies reveal the early onset of sexual maturation in females occurring at maternal doses between 2.4 and 50 µg/kg/day.[13] For example, Dr. Kembra Howdeshell and her colleagues found that when a pregnant mouse was given an extremely small bisphenol A dose of 2.4 µg/kg/day, their female offspring tended to grow larger and ovulate earlier (i.e., signs of early puberty).[14] A Japanese lab confirmed these findings in 2002.[15]

Bisphenol A Exposure May Lead to Obesity and Diabetes

A study by Dr. Beverly Rubin and her colleagues at Tufts University Medical School showed that bisphenol A makes rodents grow larger after they are exposed in the womb, confirming similar findings from previous studies.[17] When rats were fed 100 µg/kg/day of bisphenol A during pregnancy through lactation, their offspring were notably heavier after birth and into adulthood.

Significantly, in the female offspring, the lower of the two bisphenol A doses used in the study produced a larger and more persistent effect on body weight relative to the higher dose. In addition, the fact that the effect persisted long after exposure for the female offspring suggests that bisphenol A may increase the number of fat cells in the rats and predispose them to heavier weight throughout life.

In 2002, a team of researchers at the Ehime College of Health Science in Japan discovered that bisphenol A can increase the conversion of embryonic cells into fat cells.[18] In the body, this effect could result in larger numbers of fat cells developing. In addition to converting to fat cells, treated cells increased their fat content by 150 percent over 11 days. Combined with insulin, bisphenol A increased the fat content of cells by 1300 percent. In other words, this experiment documented that bisphenol A could trigger and promote the two main processes in developing obesity. In 2004, another study confirmed these findings, showing that bisphenol A alone and with insulin increased the uptake of sugar into fat cells.[19]

A recent study by Dr. Paloma Alonso-Magdalena and her colleagues showed that low-level, chronic exposure of adult mice to 10 µg/kg/day of bisphenol

U.S. Environmental Protection Agency's Current Safety Threshold for Bisphenol A

The current safety threshold established by the U.S. EPA—called the reference dose (i.e., safe dose)—was set based on animal experiments conducted prior to 1988 showing that 50 milligrams per kilogram of body weight caused weight loss in rodents. U.S. EPA declared 50 mg/kg/day the lowest observed adverse effect level, or LOAEL. To arrive at the current reference dose, U.S. EPA assumed without further study that a dose 1000 times lower than the LOAEL (i.e., 50 micrograms per kilogram per day, or 50 µg/kg/day) would be an acceptable reference dose. As over 40 studies now illustrate, the official reference dose of 50 µg/kg/day is well above the levels at which adverse affects have been found in numerous animal studies over the past decade.

For example, Dr. Kembra Howdeshell and her colleagues found that the female offspring of pregnant mice fed bisphenol A at the low dose of 2.4 micrograms per kilogram per day experienced the early onset of puberty.[16] If U.S. EPA were to use 2.4 µg/kg/day as a LOAEL and apply the same logic used to establish the current standard, the reference dose would be 2.4 nanograms per kilogram per day (ng/kg/day). A reference dose of 2.4 ng/kg/day would eliminate commercial uses of bisphenol A in food and beverage containers and products that babies are likely to put in their mouths.

A caused insulin resistance, which is a precursor to Type II diabetes in people as well as hypertension and cardiovascular disease.[20] Dr. Alonso-Magdalena's study showed that even a single dose of bisphenol A at levels currently found in humans can result in altered levels of blood glucose and insulin, and twice-daily exposure for just four days results in insulin resistance.

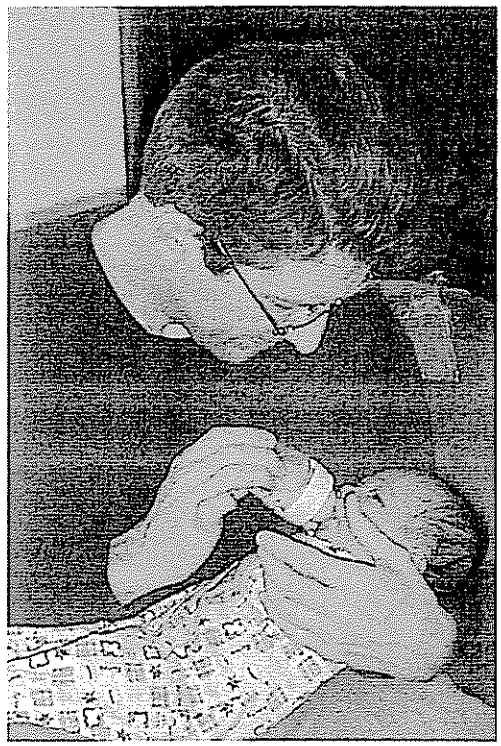
Several studies show an increased rate of postnatal growth in both males and females as a result of maternal doses between 2.4 and 500 µg/kg/day.[21] Accelerated postnatal growth is associated not just with obesity but with insulin-resistant diabetes, hypertension, and heart disease as well.

Figure 2: Rising Obesity Trend in Adolescents[22]

	Children 6-11 Years	Adolescents 11-19 years
1972	4%	6%
1978	6%	5%
1991	11%	10%
2000	15%	15%

Bisphenol A Exposure Leads to Impaired Brain Development

In most studies, bisphenol A has been found to mimic the actions of estrogen in developing neurons. In specific areas of the brain, however, bisphenol A can have the paradoxical effect of *inhibiting* the activity of estrogen, which normally increases the growth and regulates the viability of connections between neurons. In this regard, bisphenol A is similar to the breast cancer drug tamoxifen, which stimulates estrogenic responses in some tissues and inhibits estrogenic responses in other tissues. The concern



relating to this inhibitory effect of bisphenol A is that this type of disruption is associated with impaired learning and memory.[23]

Whether bisphenol A is mimicking or inhibiting estrogen, bisphenol A appears to trigger steps important in the development of the brain at the wrong times or encourages improper connections in the brain to be made. Mounting evidence from the last several years shows that bisphenol A alters brain development, leading to a number of different potential problems, including:

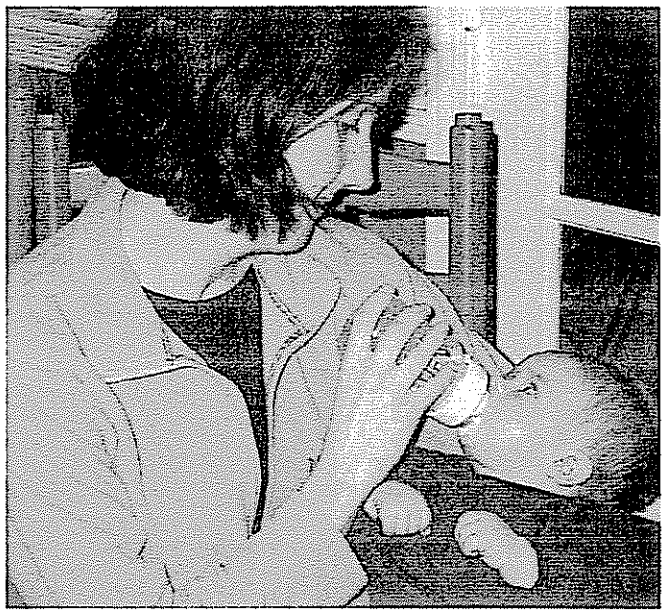
- *Hyperactivity*: Dr. Masatoshi Morita and his colleagues at the Japanese National Institute for Environmental Studies reported that a single 30 µg/kg/day bisphenol A dose given to a 5-day old rat lead to hyperactive behavior.[24] The scientists also found that bisphenol A exposure changed how the dopamine signaling system developed in brain cells, resulting in

less dopamine receptors and transporters. Dopamine is an important transmitter of nerve signals in the brain, and loss of neurons that produce dopamine occurs in Parkinson's disease.

- *Increase in aggression*: At doses between 2 and 40 µg/kg/day, fetal exposure to bisphenol A led to increased aggressive behavior by male mice, which could not be attributed to an elevation in testosterone concentration.[25]
- *Changes in response to painful or fear-provoking stimuli*: Dr. Anna Maria Aloisi and her colleagues injected pregnant and lactating rats with 40 µg/kg/day of bisphenol A. The scientists found that exposure to bisphenol A during these times modified the activity of neural pathways and changed the rats' perception of pain.[26]
- *Impaired learning and memory*: Male offspring of rats exposed to 0.1 mg/kg/day of bisphenol A consistently failed to avoid electrical shocks at a significantly increased rate compared with the control offspring, revealing that bisphenol A exposure during brain development had resulted in impaired memory.[27]
- *Reversal of normal sex differences in the brain structure and elimination of sex differences in behavior*: At 30 µg/kg/day, exposure to bisphenol A before birth and during nursing reversed the sex differences between male and female rats in an area of the brain—the locus coeruleus—which is believed to be a key brain center for anxiety and fear and is normally larger in females than in males. Exposure also eliminated the usual sex differences found in tests used to quantify both exploratory behavior and fear response.[28]
- *Decreased maternal behavior*: Dr. Paola Palanza and her colleagues exposed female mice to bisphenol A at the 10 µg/kg/day level and measured several different characteristics of maternal behavior. Some of the mice were exposed while in the womb by feeding their pregnant mothers. Some were exposed in adulthood while lactating. Others were exposed both in the womb and during adulthood. The scientists' findings showed that maternal behavior was altered in a number of ways. Females exposed to bisphenol A only as fetuses or only as adults exhibited lower levels of nursing behavior toward their offspring, increases in time resting away from offspring, and increases in time spent out of the nest. In most measurements, females exposed both in the womb and as adults did not differ from controls.[29]
- *Altered play and other socio-sexual behaviors*: At 40 µg/kg/day, the male and female offspring of rats fed bisphenol A from conception to weaning led to a masculinization of female behavior in two behavioral categories—play with females and sociosexual exploration (i.e., genital and body sniffing).[30]

Bisphenol A May Lead to Impaired Immune Function

Several studies show that altered immune function occurs at doses of bisphenol A between 2.5 and 300 µg/kg/day.[31] These studies show that immune responses may be augmented as a result of either prenatal or postnatal exposure to bisphenol A.



Bisphenol A is Linked to Increased Cancer Cell Growth

Breast cancer

A recent study showed that prenatal exposure to bisphenol A causes mammary gland cancer in adult rats.[32] Prior research had shown that bisphenol A altered the growth of mammary tissues in ways that increase the risk of breast cancer and increase the sensitivity of breast tissue to cancer causing agents.[33] In one of these earlier studies, scientists exposed mouse fetuses to doses of 25 and 250 ng/kg/day—2,000 times lower than the amount deemed safe by the U.S. EPA for humans in the U.S—causing increased breast tissue development.[34] Higher density breast tissue is a risk factor for cancer.[35]

In the most recent study by Dr. Ana Soto and her colleagues, prenatal exposure of both rats and mice to bisphenol A at doses ranging from 0.25 to 25 µg/kg/day lead to the formation of mammary gland cell growth patterns that are considered to be the precursors of breast cancer.[36]

Prostate disease and cancer

Along with breast cancer, low-dose exposure to bisphenol A is implicated in prostate cancer, as it can significantly increase prostate size. Several studies show an increase in prostate size due to hyperplasia in male mouse offspring caused by very low maternal doses of bisphenol A.[37] A more recent study shows that exposure to a very low dose of bisphenol A for just a few days after birth predisposes male rats to develop prostate cancer in adulthood.[38]

In addition to causing prostate cancer, bisphenol A can interfere with traditional methods for treating prostate cancer. In a study by Dr. Yolanda Wetherill and her colleagues, bisphenol A stimulated human prostate cancer cells, which would interfere with the standard hormone treatment used to force prostate cancer into remission.[39] This effect occurred at exactly the concentration of bisphenol A present in over 95 percent of people in the U.S according to the U.S. Centers for Disease Control and Prevention.

Bisphenol A is Associated with Sperm Defects

In 1998, Dr. Frederick vom Saal and his colleagues at the University of Missouri at Columbia published one of the first studies linking reduced sperm production with bisphenol A exposure. The scientists fed bisphenol A to female rats at a dose of 20 µg/kg/day for six days during pregnancy. They found that males born to exposed rats produced 20 percent less sperm after they matured than normal males.[40] They also found that treated offspring had physical changes in hormone-secreting glands not found in untreated mice, even at a dose 10 times smaller.

A few years later, Dr. Motoharu Sakaue and his colleagues in Japan added to these findings, discovering that bisphenol A reduces the number of sperm in rats, even when given doses after puberty.[41] After feeding small doses to rats (20 µg/kg/day for six days at week 13 of life), they noted a generalized decline in the ability of treated rats to produce sperm. The scientists concluded that bisphenol A retarded the development of germ cells that normally takes place as the male rat's reproductive system matures from week 14 to week 18. The scientists further concluded that the effects occurred in a dose range "relevant to the daily level of exposure in man."

Figure 3: Average Decline in Sperm Density Across North America and Europe in the 20th Century[42]

	Sperm Density (million/ml)	
Year of Sample Collection	North America	Europe
1934	108	
1945		169.5
1996	59	58
Decline in Sperm Density	45%	66%

A 2002 study also found lowered sperm production as a result of bisphenol A exposure, in addition to other problems. Adult male mice having ingested 5 to 100 µg/kg/day of bisphenol A showed a significant reduction in testicular sperm counts, the efficiency of sperm production, and the weight of the testes.[43] According to the study's authors, the "results suggest that male fertility and reproduction is impaired by bisphenol A."

Additional studies have confirmed the 2002 findings of reduced testes weight. One lab demonstrated that fetal exposure to bisphenol A causes reduced testes weight at concentrations found in humans.[44] Another found that bisphenol A-treated rats had a significant decrease in the weight of the testes in addition to a reduction in sperm motility and sperm count.[45]

Bisphenol A is Linked to Impaired Female Reproductive Development

In 2002, evidence of impaired female reproductive development as a result of bisphenol A exposure was published. Pregnant rats given 0.1 mg/kg/day of bisphenol A gave birth to female offspring with vaginal deformations, apparently caused by a disruption of the estrogen signal required for normal development.[46]

Bisphenol A Exposure May Lead to Miscarriage

Low-dose bisphenol A exposure is also associated with miscarriages in women.[47] In one recent study, scientists found levels of bisphenol A three times higher in women with a history of recurrent miscarriage than in women who had normal pregnancies.[48] The results of this study were predicted by an earlier study by Dr. Hunt and her colleagues that found bisphenol A causes meiotic aneuploidy in mice, a condition that is the largest known cause of spontaneous miscarriage in people.

Animal Studies Predict Human Health Outcomes

Although a few studies on bisphenol A rely on human data, most studies on the effects of bisphenol A exposure involve laboratory animal experiments. At this time there is inadequate information to determine whether the absorption, distribution, and excretion of bisphenol

A is identical in rodents and humans. There is extensive evidence, however, that the sensitivity of tissues to bisphenol A in the animals used in experiments is virtually identical to the sensitivity of human tissues to bisphenol A.

Indeed, the U.S. government has concluded that animal studies are a vital guide to identifying health risks for humans.[49] And, it is clear from a large number of studies that the concentration of biologically active bisphenol A in the blood, tissues, and urine of the average person is higher than levels that cause harm due to administration of the doses in the animal experiments described above.

Because the chemical bond between bisphenol A molecules in polycarbonate plastic is unstable, the plastic can degrade over time and leach bisphenol A into materials with which it comes into contact. As a result, exposure to bisphenol A is widespread.

Bisphenol A Levels in Humans are Above Harmful Levels Found in Studies

According to the U.S. Centers for Disease Control and Prevention (CDC), 95 percent of Americans have detectable levels of bisphenol A in their bodies.[50] In a recent CDC study, the observed bisphenol A levels detected—0.1 to 9 ppb—were at and above the concentrations known to reliably cause adverse effects in laboratory experiments. Of significant concern, the median bisphenol A level in human blood and tissues, including in human fetal blood, is higher than the level that causes adverse effects in rodents.[51]

Despite the fact that bisphenol A is metabolized by the body, the CDC's findings provide strong evidence that exposure to the chemical is very frequent or nearly continuous. Otherwise, over 95 percent of the people examined would not have had detectable bisphenol A in this relatively high range. While 0.1 to 9 ppb may not seem like a high concentration, one recent study found significant increases in calcium inflow even at the lowest levels of bisphenol A exposure in the low part per trillion (ppt) range.[52] Increases in calcium within the cell initiate a wide array of processes within the cell such as regulating hormone secretion and controlling gene activity. The CDC data show that people contain BPA in the *parts per billion* level—1,000 times higher than the lowest exposure at which an effect was seen on calcium influx.

The CDC's findings are confirmed by numerous studies conducted in other countries showing virtually identical levels of bisphenol A in blood and tissues collected from human fetuses and adults.[53] These findings suggest that human exposure to significant amounts of bisphenol A must be continuous and via multiple sources.[54]

How Bisphenol A Gets into Our Body

Bisphenol A leaches into our bodies through our everyday contact with household products containing the chemical. The following have all been shown to result in an increase in the rate of leaching of bisphenol A:[55]

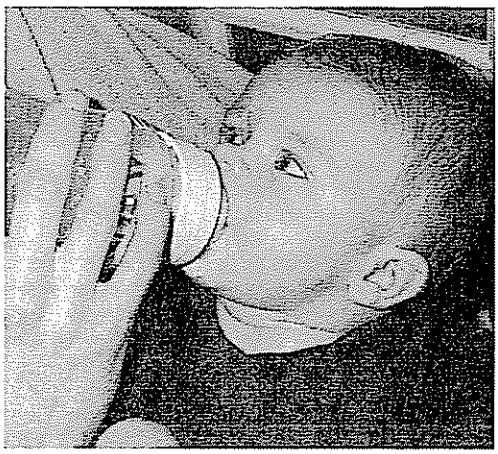
- the presence of acidic or basic food or beverages stored in cans lined with epoxy resin containing bisphenol A or in polycarbonate plastic;
- heating of polycarbonate plastic containers; and
- repeated washing of polycarbonate products.

Another potential source of human exposure to bisphenol A is through water used for drinking or bathing.[56] This is because bisphenol A contamination is widespread in the environment. For example, bisphenol A can be measured in rivers and estuaries at concentrations that range from under 5 ng/L (5 ppt) to over 1900 ng/L (1.9 ppb). Sediment loading can also be significant, with levels ranging from under 5 ppb to over 100 ppb.[57] Moreover, studies conducted in the U.S. and Japan have shown that bisphenol A accounts for the majority of estrogenic activity that leaches from landfills into the surrounding ecosystem.[58]

Report Findings: All Baby Bottles Tested Leach Bisphenol A

Do all baby bottles leach bisphenol A? To answer this question, we analyzed five of the most popular brands of baby bottles to determine whether bisphenol A leaches into liquid with which it comes into contact. Laboratory tests found that all five bottle brands leached levels of bisphenol A exceeding the levels found to cause harm in scientific studies.

As described above, bisphenol A is the building block for polycarbonate plastic. Polycarbonate plastic is a very hard, unbendable plastic and typically clear in appearance. It should be noted, however, that some polycarbonate plastic is colored with bright colors. Polycarbonate plastic baby bottles can be distinguished from bottles made of other types of plastic—primarily polypropylene-based plastic—because the latter is squeezable and typically opaque in appearance. Also, polycarbonate plastic bottles often have the number "7" in the recycling triangle on the bottom of the bottle and, in some cases, the letters "PC" next to the recycling triangle. The five bottle brands we tested were made from polycarbonate plastic.



The five bottle brands we tested are a sample of the bottles on the market and are not intended to represent a comprehensive list. We did, however, rely on data collected from an extensive parent survey to determine the most popular brands of bottles to test. For more information, refer to Appendix A.

This section describes the baby bottle brands testing positive in the lab for bisphenol A leaching. Appendix A reports the specific type of bottle by brand and the level of bisphenol A found to leach from each product. The presence of bisphenol A at any level in baby bottles is cause for concern, as there is no safe level.

These findings are clearly alarming for parents and others who care about the health and safety of their children. Unfortunately, parents do not have the information they need to ensure the products they purchase do not contain toxic chemicals. In "Recommendations for Parents," later in this report, we give parents some tips they need in order to begin to protect their children. Parents will be unable to fully protect their children, however, without adequate action by policymakers. We list these actions in "Recommendations for Policymakers."

Polycarbonate Baby Bottles Leach Bisphenol A

Numerous laboratory studies show that polycarbonate plastic breaks down and leaches bisphenol A into food or beverages in contact with the plastic.[59] Bisphenol A molecules are bound by ester bonds to form a polymer used

to make polycarbonate plastic. These studies demonstrate the instability of the chemical bond between bisphenol A molecules. The instability causes the polymer to decay with time and bisphenol A to be released into materials with which it comes into contact.

Polycarbonate baby bottles are no different. In a 2003 study conducted in Norway, bisphenol A leaching was detected in 12 polycarbonate baby bottles subjected to simulated use—dishwashing, brushing, and boiling. Levels of bisphenol A detected in liquids held in these bottles exceeded 8 ppb.[60]

Our study confirmed the findings of the Norway study. We tested five popular baby bottle brands. All five bottles leached bisphenol A at varying levels in the same range detected in the Norway study (Table 1).

Table 1: Summary of Testing for Bisphenol A Leaching in Baby Bottles

Baby Bottle Brand	Range of Bisphenol A Detected (parts per billion)
Avent	8 – 10 ppb
Dr. Brown's	6 – 7 ppb
Evenflo	8 – 9 ppb
Gerber	6 – 7 ppb
Playtex	5 – 6 ppb

Natural Feeding Bottle by Avent:

Testing Found Leaching of Bisphenol A at 8-10 ppb Level

Classic by Evenflo:

Testing Found Leaching of Bisphenol A at 8-9 ppb Level

Natural Flow by Dr. Brown's:

Testing Found Leaching of Bisphenol A at 6-7 ppb Level

Premium Feeding System by Gerber:

Testing Found Leaching of Bisphenol A at 6-7 ppb Level

VentAire by Playtex:

Testing Found Leaching of Bisphenol A at 5-6 ppb Level

Alarmingly, all five polycarbonate plastic bottles leached bisphenol A at levels found to cause harm in numerous animal studies evaluating various health effects from exposure to the chemical. Although consumers can try to avoid polycarbonate plastic bottles, most parents are unaware that toxic chemicals can leach from these products. Rather than put the burden on consumers, California and the U.S. should do more to protect its children by banning such products from store shelves.

The Government Fails To Protect Consumers From Toxic Chemicals

Many people think, incorrectly, that the government would prohibit chemicals from entering the market if they were not safe. In truth, the regulatory process has failed to work the way the public believes it should.

Chemicals Enter the Market Before Being Proven Safe for Human Health

The U.S. government's regulation of chemicals is based on the presumption that chemicals are innocent until they are proven to harm human health or the environment. This presumption is startling, especially when you consider:

- There are an estimated 80,000 chemicals registered for commercial use in the U.S.[61]

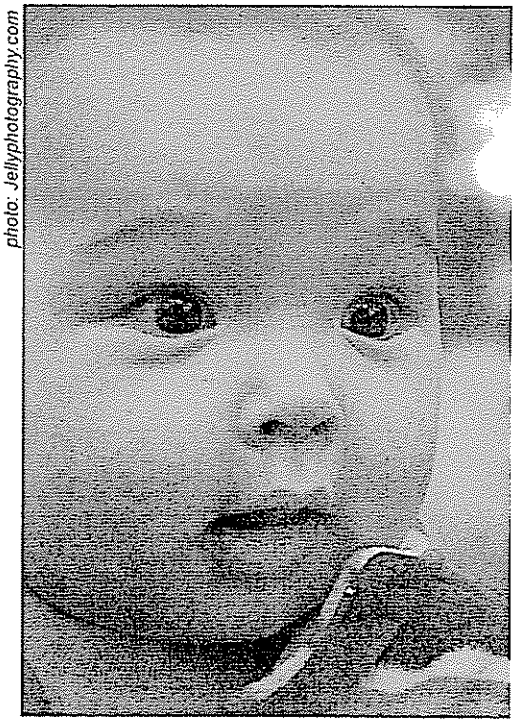


photo: Jellyphotography.com

- Only a very small percent of these chemicals have been tested for safety to human health.[62]
- An estimated 2,000 new chemicals are introduced each year, or an average of seven new chemicals each day.[63]

In 1976, Congress passed the primary law regulating toxic chemicals in the U.S., the Toxic Substances Control Act (TSCA), which grandfathered all existing chemicals on the market into use without health-effects testing or analysis.[64] Most of these chemicals emerged in the 1940s and 1950s when few laws governed chemical safety.

TSCA divides all the chemicals on the market into two categories: existing chemicals and new chemicals. Existing chemicals are chemicals on the market as of 1979. These make up approximately 99 percent by volume of the chemicals on the market today.[65] Existing chemicals are considered safe unless U.S. EPA can establish that: 1) they will in fact present an unreasonable risk to human health or the environment, 2) the agency is choosing the least burdensome regulation to reduce risks to a reasonable level, and 3) the benefits of regulation outweigh the costs to industry.[66] Such a high burden has essentially paralyzed the U.S. EPA from regulating or restricting chemicals predating 1980.

Companies that wish to introduce new chemicals to the U.S. market must notify U.S. EPA at least 90 days before producing or importing a new chemical. However, TSCA only requires that manufacturers submit health-effects testing information that is "in their possession," thereby creating a disincentive for manufacturers to conduct any

testing.[67] In fact, the U.S. EPA reports that the vast majority of pre-market notices by manufacturers contain no information on health or environmental impacts.[68]

Throughout its 30-year history, TSCA has rarely been amended, yet it clearly fails to effectively regulate toxic chemicals. Since the law's inception, U.S. EPA has never used its authority to ban a chemical and has only formally regulated five different chemicals, including polychlorinated biphenyls (PCBs), which Congress ordered regulated through TSCA. U.S. EPA's lax regulation can be attributed to the unreasonably high burden of proof the law places on the agency to show that a chemical poses an unreasonable risk to human health or the environment.

Numerous studies—including those conducted by the National Academy of Sciences, the U.S. General Accounting Office, the Congressional Office of Technology Assessment, and the U.S. EPA—have concluded that TSCA does not provide an effective means for assessing the hazards of chemicals or controlling those of greatest concern.[69]

U.S. EPA should be able to guarantee that chemicals on the market are safe for human health and the environment. The agency estimates the cost for a full round of basic screening tests, including tests for reproductive and developmental toxicity, at about $205,000 per chemical.[70] Although these tests have been conducted for a limited number of chemicals, we need this basic information for all chemicals currently in use. The chemical industry, with profits in excess of $45 billion in 2005, should pay this price to protect both human health and the environment.[71]

Regrettably, California relies on the federal government's failed regulatory system to protect its residents from chemicals used in commerce. California has no regulatory framework for reviewing chemicals prior to their introduction on the market and use in consumer products.

Labels are not Required for Consumer Products Even if They Contain Potentially Hazardous Chemicals

Because chemicals are not sufficiently tested and regulated before they enter into commerce, manufacturers of consumer products often use chemicals with unknown—and in some cases, known—health hazards to make products ranging from children's toys to medical devices. In some cases, manufacturers of consumer products have no information on whether the chemicals they are using to make their products are harmful. In other cases, scientific evidence shows that a chemical used in a particular product may be harmful.

Just as the law fails to require chemical manufacturers to prove the safety of their chemicals, the law fails to require adequate warning for consumers even when scientific evidence shows that a chemical used in a particular product may be harmful. For example, extensive scientific evidence shows that bisphenol A may be harmful to human health. Yet, manufacturers of baby bottles and other products containing bisphenol A are not required to label their products as containing bisphenol A.

Proposition 65, which is a law passed by voters in 1986, requires California to establish and update a list of chemicals known to the state to cause cancer or reproductive toxicity.[72] One major provision of the law requires that "clear and reasonable" warnings be provided for listed chemicals if exposure would

exceed the maximum allowable level designated by the state. Importantly, exposures at any level above the maximum allowable level are permitted by the law as long as an appropriate warning is provided.

Proposition 65 warnings are required in a variety of contexts, including for various consumer products, discharges from manufacturing or distribution facilities, and exposures that may occur as a result of entering or residing in certain buildings. For consumer products, the law does not require that an individual product be labeled. The law simply requires that a warning be "clear and reasonable," which could include labeling but also permits the posting of notices. Moreover, Proposition 65 warnings do not identify the chemical or chemicals to which the warning refers, nor do they provide any information on levels of exposure that are expected to occur as a result of using the product or the potential hazards associated with those levels of exposure.

Although Proposition 65 has served as an incentive for some manufacturers to reformulate their products, it does not provide consumers with sufficient information to make better choices about products that are safe for their children.

Consumers have the right to know whether products they use every day contain chemicals that are known or have the potential to cause harm to them or their families. And they need enough specificity about an individual product to be able to properly evaluate whether they should avoid particular products.

Recommendations For Policymakers

Parents cannot be expected to track the thousands of potentially harmful toxic chemicals they and their families come into contact with every day. In light of the federal government's failure to adequately protect human health, California must act to adequately protect those most vulnerable in its population. Parents should call on policymakers to take the following actions.

Phase Out Hazardous Chemicals

Based on the weight of the scientific evidence showing the harm caused by exposure to bisphenol A, California should act now. Given that data from the U.S. Centers for Disease Control and Prevention show that bisphenol A is present in humans at levels found to be harmful in laboratory studies, California should phase out the use of bisphenol A, especially in products used by children.

In the absence of both federal and state action, the city of San Francisco has already taken steps to protect children's health. In June 2006, San Francisco passed a prohibition on the use of bisphenol A in toys and child care articles intended for use by children under the age of three.[73] California will likely consider similar legislation this year.

Label Products Containing Hazardous Chemicals

Parents currently have little information to inform their decisions when purchasing products for their family. With no government-mandated labels on products and no ability to readily gain information about the ingredients used in a product, parents are left in the dark as to how they can best protect their children. The first step to protecting children is to give parents the tools they need to make safer choices. Manufacturers should be required to label children's products if they contain a chemical that is either known to be hazardous or has the potential to be hazardous. In addition to listing the hazardous or potentially hazardous ingredient, the specific health risks associated with the chemical should be described on the product.

Reform Chemicals Policy

In order to better protect human health and the environment, California must adopt strong chemicals policies. First, chemical manufacturers should be required to provide all hazard and health-effects information to the government so agencies can begin to assess the thousands of chemicals currently on the market for which little or inadequate data are available. Next, pre-market hazard and health-effects testing should be required for all new chemicals before they are introduced into commerce. Finally, the California Environmental Protection Agency must have the authority to ban or restrict the use of a chemical if it can harm human health. To that end, California must establish a regulatory framework for regulating chemicals in commerce without the legal barriers that make the federal Toxic Substances Control Act ineffective.

Recommendations For Parents

A few small, easy changes in the products you buy and use can help reduce your child's exposure to toxic chemicals.

At the Store

Choose safer toys and teethers.

- Look for "PVC-free" on the labels of soft plastic toys and teethers. Another class of chemicals shown to disrupt the hormone system—phthalates—is found in polyvinyl chloride (PVC) plastic. PVC plastic is used to make different types of children's products, including some teethers and soft plastic toys. Some manufacturers have removed PVC from their children's products, especially products intended to be put into children's mouths. Unfortunately, no law requires or regulates these labels, and few products are labeled as such. When parents have a question about the chemicals in a product, they should call the manufacturer.
- Choose wooden toys. There are countless manufacturers of high quality wooden toys in the market. Everything from baby rattles to kitchen play-sets are now made out of wood. Some commonly available brands include Plan Toys, Haba, Turner Toys, Selecta, and Holztiger.

Choose safer food packaging and serving containers.

- Avoid polycarbonate plastic in food containers. Check the bottom/underside of the product. If you see "PC" (usually in or near the recycling triangle) signifying polycarbonate plastic, do not purchase it. Often a number "7" on the bottom in the recycling triangle, by itself, also means the material is polycarbonate, but not always. To be safe, avoid #7 plastic. Choose plastics labeled #1, #2, or #5 in the recycling triangle, but do not heat beverages or food in plastic containers of any kind.
- Avoid PVC plastic in food containers. Check the bottom/underside of the product. If you find the number "3" in the recycling triangle, it is made from PVC plastic and should be avoided. Choose plastics labeled #1, #2, or #5 in the recycling triangle, but do not heat beverages or food in plastic containers of any kind.
- Avoid canned foods: Unfortunately, bisphenol A can leach from metal can lining into the foods and liquids contained within. Buy baby food in glass containers, and avoid feeding your child food from cans as much as possible. You can often find popular children's foods, such as tomato sauce, applesauce, and black beans, in glass jars.
- Choose safer containers for sippy cups and water bottles. Look for plastics labeled #1, #2, or #5 in the recycling triangle. As an alternative to hard plastic water bottles (such as the polycarbonate Nalgene bottles), try a lightweight stainless steel bottle instead.
- Choose glass or safer-plastic baby bottles. Almost all plastic baby bottles are made from polycarbonate plastic containing bisphenol A, but they are rarely labeled as such. With as few as 50-100 washings—even before you see wear—significant amounts of bisphenol A can leach into your baby's

milk. For the best protection, switch to using glass bottles for all or most of baby's use. Contrary to claims by the plastics industry, glass bottles are extremely durable and safe (and wash well in the dishwasher). And after all, they were good enough for you when you were a baby! Evenflo is one of the only glass bottle makers around (some Babies "R" Us stores carry them and they are available on-line). A couple of manufacturers make their baby bottles from a safer polypropylene-based plastic (a softer, opaque plastic), which has not been associated with the developmental problems linked to bisphenol A.

- **Choose metal feeding utensils and enamel or ceramic plates.** While many manufacturers have removed phthalates from products intended to be put into young children's mouths, without a law prohibiting their use, there is no guarantee that these products, such as soft, plastic-coated feeding spoons, are made without phthalates. Look for PVC-free labels or buy stainless steel, enamel, ceramic, or glass. (Note that enamel cannot be put in the microwave, and you should not use old pottery that could have lead-based glazes).
- **Avoid foods wrapped in plastic.** Almost all commercial grade plastic cling wrap contains PVC plasticized with phthalates, and other plastic food packaging may be made of PVC, as well. Avoid buying foods wrapped in plastic, especially cheeses and meats. Buy deli-sliced cheeses and meats and have them wrapped in paper. If you can't avoid buying plastic-wrapped foods, cut off a thin layer of the cheese or meat when you get home and store the remainder in glass or less-toxic plastic.

At Home

- **Use glass to heat food or liquid in the microwave. You should not heat food in plastic containers or on plastic dishware, or heat liquids in plastic baby bottles.** Heating food and liquids in plastic containers can cause chemicals and additives in the plastics to leach out more readily—right into baby's food and milk. While some plastic containers are marketed as "microwave safe," it is safest to avoid them for heating.
- **If you do use plastic bottles, containers, or dishware, avoid harsh detergents or hot water when washing them to reduce exposure.** Do not put plastic bottles, containers, or dishware in the dishwasher. Also, throw out any plastic bottles, containers, and dishware that start to look scratched or hazy. Do not let milk sit for long periods of time in plastic.
- **Avoid letting your child put plastic toys in his/her mouth.** Toys designed for older children are more likely to contain phthalates or bisphenol A. It is assumed that young children will not mouth these toys—such as action figures and Barbie dolls. To be safe, keep all plastic toys out of children's mouths. Call the manufacturer if you want to know if a product contains phthalates or bisphenol A.

METHODOLOGY

Based on a 2005-06 consumer survey that we conducted of over 2,800 parents, we selected the most popular baby bottle brands to test for the presence of bisphenol A. Respondents of the survey were given a list of 11 brand names from which to select the brand or brands they used with their children. The top five brands used by the respondents included: Avent, Dr. Brown's, Evenflo, Gerber, and Playtex. For this study, we tested bottles from these five brands to determine the amount of bisphenol A that leaches into liquid contained therein.

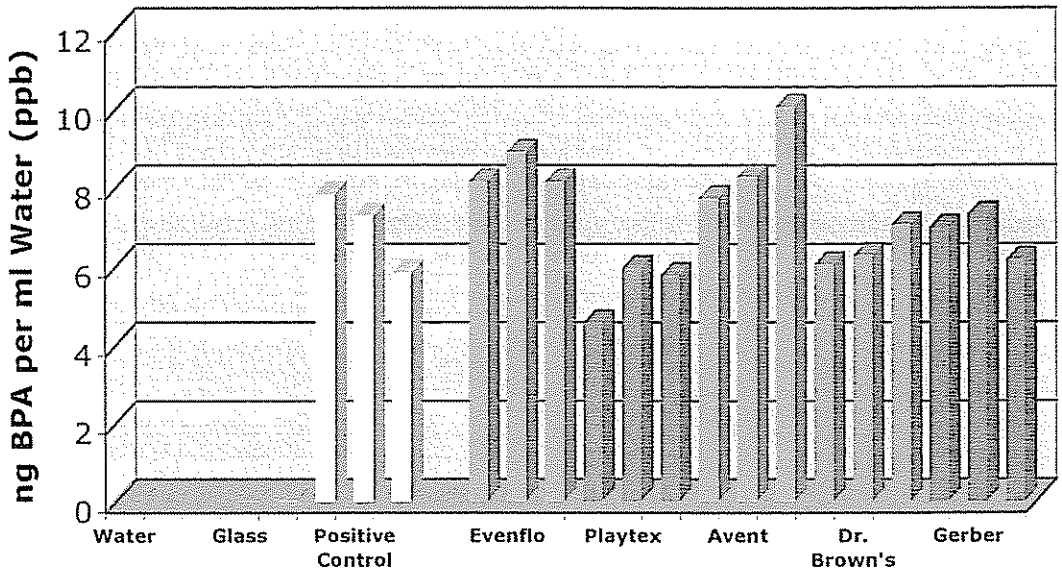
To ensure the reproducibility of the results, we tested three of each baby bottle brand. We bought each of the three bottles from a different retailer in California to avoid the possibility that the bottles were from the same stock. We purchased the bottles from four popular retailers in the state, patronizing multiple locations of two of the retailers.

We provided funds to conduct a research project at a lab (XenoAnalytical) at the University of Missouri-Columbia to measure bisphenol A at environmentally relevant concentrations of leaching from baby bottles. The investigators at the University of Missouri were chosen for this project because they were the first to predict and measure the low-dose effects of bisphenol A in animal studies and had also previously reported findings concerning leaching of bisphenol A from polycarbonate.

The lab used published procedures with sensitivities below 0.01 nanograms (ng) per milliliter (ml) of bisphenol A in water (0.01 ppb bisphenol A in water) by high-performance liquid chromatography (HPLC) with CoulArray detection, and multiple-point standard curves over the full range of sample values. The purified water used to extract bisphenol A from the bottles contained bisphenol A at less than 0.01 ng/ml. The assay of the baby bottles included three negative and three positive control bottles. The negative controls were glass baby bottles to control against introduction of bisphenol A during any technical handling. The positive controls were Lexan(r) polycarbonate sport water bottles known to release bisphenol A in prior studies; positive controls were to confirm effectiveness of the leaching procedure. The bisphenol A determination by HPLC was confirmed by estrogenic activity in an estrogen-sensitive cell culture bioassay, and the estrogenic activity was confirmed by inhibition with an antiestrogen.

The assays were conducted on the 15 polycarbonate baby bottles, three glass baby bottles as negative controls against introduction of bisphenol A in water or technical handling, and three polycarbonate sport water bottles known to release bisphenol A from prior studies as positive controls to confirm effectiveness of the leaching procedure. All bottles were incubated with water at 80 degrees centigrade for 24 hours to simulate 50 to 75 dishwashing cycles using the sanitize cycle. The range of bisphenol A detected in the 15 polycarbonate baby bottles was 4 to 10 parts per billion (ppb), while the bisphenol A extracted from the negative control glass baby bottles was less than 0.05 ng/ml (Figure 4, next page). The levels detected in the 15 polycarbonate baby bottles varied based on the brand tested, as detailed in Appendix A.

Figure 4: Bisphenol A Extracted from Polycarbonate Baby Bottles



APPENDIX A

Bottle Brand	Type	Product Number	Bisphenol A Level Detected (ppb) (Bottle #1)	Bisphenol A Level Detected (ppb) (Bottle #2)	Bisphenol A Level Detected (ppb) (Bottle #3)
Avent	Natural Feeding Bottle (9 oz)	UPC Code: 61269 00230	7.74	8.29	10.07
Dr. Brown's	Natural Flow (8 oz)	UPC Code: 72239 00250	6.07	6.29	7.07
Evenflo	Classic (8 oz)	UPC Code: 42700 12113	8.17	8.91	8.16
Gerber	Premium Feeding System (9 oz)	UPC Code: 15000 78715	6.99	7.34	6.18
Playtex	VentAire (9 oz)	UPC Code: 78300 01162	4.58	5.96	5.79

NOTES

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[73] San Francisco Ordinance No. 120-06 (2006).

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Exhibit C

An Extensive New Literature Concerning Low-Dose Effects of Bisphenol A Shows the Need for a New Risk Assessment

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Bisphenol A (BPA) is the monomer used to manufacture polycarbonate plastic, the resin lining of cans, and other products, with global capacity in excess of 6.4 billion lb/year. Because the ester bonds in these BPA-based polymers are subject to hydrolysis, leaching of BPA has led to widespread human exposure. A recent report prepared by the Harvard Center for Risk Analysis and funded by the American Plastics Council concluded that evidence for low-dose effects of BPA is weak on the basis of a review of only 19 studies; the report was issued after a delay of 2.5 years. A current comprehensive review of the literature reveals that the opposite is true. As of December 2004, there were 115 published *in vivo* studies concerning low-dose effects of BPA, and 94 of these report significant effects. In 31 publications with vertebrate and invertebrate animals, significant effects occurred below the predicted "safe" or reference dose of 50 μg/kg/day BPA. An estrogenic mode of action of BPA is confirmed by *in vitro* experiments, which describe disruption of cell function at 10^{-12} M or 0.23 ppt. Nonetheless, chemical manufacturers continue to discount these published findings because no industry-funded studies have reported significant effects of low doses of BPA, although > 90% of government-funded studies have reported significant effects. Some industry-funded studies have ignored the results of positive controls, and many studies reporting no significant effects used a strain of rat that is inappropriate for the study of estrogenic responses. We propose that a new risk assessment for BPA is needed based on *a*) the extensive new literature reporting adverse effects in animals at doses below the current reference dose; *b*) the high rate of leaching of BPA from food and beverage containers, leading to widespread human exposure; *c*) reports that the median BPA level in human blood and tissues, including in human fetal blood, is higher than the level that causes adverse effects in mice; and *d*) recent epidemiologic evidence that BPA is related to disease in women. *Key words:* bisphenol A, dose response, endocrine disruptors, low dose, nonmonotonic, risk assessment scientific integrity. *Environ Health Perspect* 113:926–933 (2005). doi:10.1289/ehp.7713 available via *http://dx.doi.org/* [Online 13 April 2005]

Bisphenol A (BPA) is a known environmental estrogen that is used as the monomer to manufacture polycarbonate plastic, the resin that is used as linings for most food and beverage cans, as dental sealants, and as an additive in other widely used consumer products. BPA is one of the highest-volume chemicals produced worldwide; global BPA capacity in 2003 was 2,214,000 metric tons (> 6.4 billion lb), with 6–10% growth in demand expected per year (Burridge 2003). Heat and contact with either acidic or basic compounds accelerate hydrolysis of the ester bond linking BPA molecules in polycarbonate and resins. Specifically, heating of cans to sterilize food, the presence of acidic or basic food or beverages in cans or polycarbonate plastic, and repeated washing of polycarbonate products have all been shown to result in an increase in the rate of leaching of BPA (Brotons et al. 1995; Consumers Union 1999; Howdeshell et al. 2003; Kang and Kondo 2002; Kang et al. 2003; Olea et al. 1996; Raloff 1999). In addition, another potential source of human exposure is water used for drinking or bathing. Studies conducted in Japan (Kawagoshi et al. 2003) and in the United States (Coors et al. 2003) have shown that BPA accounts for most estrogenic activity that leaches from landfills into the surrounding ecosystem.

Convincing evidence that there is widespread exposure to BPA is shown by the finding of Calafat et al. (2005) that 95% of urine samples from people in the United States examined by the Centers for Disease Control and Prevention (CDC) have measurable BPA levels [range, 0.4 ppb (10th percentile) to 8 ppb (95th percentile); median = 1.3 ppb]. As described by Calafat et al. (2005), these levels are consistent with findings from other countries. For example, levels of unconjugated (parent) BPA in human blood and tissues are also in the same 0.1–10 ppb range (Ikezuki et al. 2002; Schonfelder et al. 2002) detected by Calafat et al. (2005) in urine. Because there is evidence that BPA is rapidly metabolized (Volkel et al. 2002), these finding suggest that human exposure to significant amounts of BPA must be continuous and via multiple sources. A relationship between blood levels of BPA and body fat in women has been reported (Takeuchi et al. 2004).

In this commentary, we document for the scientific, public health, and regulatory communities that exposure of experimental animals to "low doses" of BPA, which result in tissue levels within and even below the range of human exposure, has been related to adverse effects in a large number of recently published studies. A recent case–control study reporting that blood levels of BPA are related to ovarian disease in women (Takeuchi et al. 2004) adds to our concern. A large number of *in vitro* studies show that effects of BPA are mediated by both genomic and nongenomic estrogen-response mechanisms, with disruption of cell function occurring at doses as low as 1 pM or 0.23 ppt (Wozniak et al. 2005). Although the focus of most studies of effects of BPA has been on its estrogenic activity, recent reports indicating the potential to disrupt thyroid hormone action (Moriyama et al. 2002; Zoeller et al. 2005) mean other modes of action must also be considered. Very low part-per-trillion doses of BPA also cause proliferation of human prostate cancer cells via binding to a mutant form of the androgen receptor expressed in a subpopulation of prostate cancer cells (Wetherill et al. 2002), although BPA acts as an androgen antagonist in the presence of the wild-type androgen receptor (Lee et al. 2003; Paris et al. 2002) and can also block testosterone synthesis (Akingbemi et al. 2004). A comprehensive document containing all of the low-dose BPA references, as well as information concerning mechanisms of action, pharmacokinetics, sources of exposure, and exposure levels in humans, is available online (Endocrine Disruptors Group 2005).

Our current conclusion that widespread exposure to BPA poses a threat to human health directly contradicts several recent reports from individuals or groups associated with or funded by chemical corporations [Association of Plastics Manufacturers in

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We thank J.P. Myers for comments during the preparation of the manuscript.

Funding during the preparation of the manuscript was provided by National Institute of Environmental Health Sciences grant ES11283 to F.S.

The authors declare they have no competing financial interests.

Received 2 November 2004; accepted 12 April 2005.

Europe (APM) 2005; Gray et al. 2004; Kamrin 2004; Purchase 2004]. For example, a recently published report on BPA prepared by a panel convened by the Harvard Center for Risk Analysis (HCRA), which was funded by the American Plastics Council (APC), concluded that "the weight of the evidence for low-dose effects is very weak" (Gray et al. 2004). However, the charge to the HCRA panel, which was to perform a weight-of-the-evidence evaluation of available data on the developmental and reproductive effects of exposure to BPA in laboratory animals, led to an analysis of only 19 of 47 available published studies on low-dose effects of BPA. The deliberations of the HCRA were in 2001–2002, and accordingly, a cut-off date of April 2002 was selected for consideration of the published literature. It is regrettable that the relevance of the analysis was further undermined by a delay of 2.5 years in publication of the report. During the intervening time, between April 2002 and the end of 2004, a large number of additional articles reporting low-dose effects of BPA in experimental animals have been published. The result is that by the end of 2004, a PubMed (National Library of Medicine, Bethesda, MD) search identified 115 published studies concerning effects of low doses of BPA in experimental animals.

The last U.S. Environmental Protection Agency (EPA) risk assessment for BPA was based on research conducted in the 1980s [Integrated Risk Information System (IRIS) 1988]. The most recent risk assessment of BPA was based on a comprehensive review of the scientific literature conducted in 1998 by the European Union, with some selected articles added through 2001, at which time few of the 115 low-dose BPA studies had been published [European Chemicals Bureau (ECB) 2003]. Below, we describe recent findings concerning mechanisms mediating effects of very low doses of BPA, the adverse effects being reported in animals, and recent findings from human studies. These published findings lead us to strongly recommend that a new risk assessment for BPA be initiated.

The Definition of "Low Dose"

The U.S. EPA considers "low-dose" effects of environmental endocrine-disrupting chemicals to refer to effects being reported for chemicals at doses lower than those used in traditional toxicologic studies conducted for risk assessment purposes. For BPA, the lowest dose studied for risk assessment purposes was 50 mg/kg/day, which is the currently accepted lowest observed adverse effect level (LOAEL) that was used to calculate a reference dose of 50 μg/kg/day based on experiments conducted in the 1980s (IRIS 1988).

BPA is often described as a very "weak" estrogen because in a few assay systems, such as MCF-7 breast cancer cells in culture, the dose of BPA required to stimulate cell proliferation (~ 10^{-7} M or 23 ppb) is roughly 100,000 times higher relative to estradiol, which stimulates cell proliferation at approximately 10^{-12} M (Welshons et al. 1999). This contrasts, however, with the stimulation by BPA of calcium influx in MCF-7 cells that was significant at the lowest dose tested, which was 10^{-10} M or 23 ppt (Walsh et al. 2005). BPA also stimulated calcium influx and prolactin secretion in rat pituitary tumor cells at the lowest dose tested (10^{-12} M or 0.23 ppt), and the magnitude of the response to BPA was similar to the response to the same dose of estradiol (Wozniak et al. 2005). It is difficult to conceive how a chemical that can alter cell function at concentrations < 1 ppt can be characterized as a "weak" endocrine disruptor.

Low-dose effects of endocrine-disrupting chemicals such as BPA are mediated by endocrine-signaling pathways that evolved to act as powerful amplifiers, with the result that large changes in cell function can occur in response to extremely low concentrations (Welshons et al. 2003). Thus, information concerning the *in vivo* potency of estradiol is critical with regard to predicting the *in vivo* bioactivity of chemicals such as BPA. *In vivo* potency of estrogenic chemicals is determined by the affinity of the chemical for the specific type of estrogen receptor (ER) that mediates the effect, the rate of absorption and metabolism, and binding of the chemical to plasma estrogen-binding proteins. The initial interest in low-dose effects of BPA was based on the observation that BPA showed limited binding to plasma estrogen-binding proteins (Nagel et al. 1997), which results in higher free plasma BPA relative to estradiol. It is well known that it is the free hormone level in blood that is predictive of biologic activity (Nagel et al. 1999). A much higher free BPA concentration in blood relative to estradiol would not be taken into account in predicting its *in vivo* potency based simply on cell culture studies conducted in culture medium.

Before conducting the first low-dose *in vivo* study with BPA, vom Saal et al. (1997) found that an increase in size of the fetal mouse prostate occurred in response to an experimental increase in free serum estradiol in fetuses of 0.1 pg/mL serum (0.1 ppt or 0.4×10^{-12} M), from 0.2 pg/mL in control fetuses to 0.3 pg/mL free serum estradiol in estrogen-exposed fetuses. Although this finding was initially controversial, other *in vivo* and *in vitro* studies have since confirmed that very low doses of the estrogenic drug diethylstilbestrol (DES) stimulate an increase in size of the fetal mouse prostate (Gupta 2000; Timms et al. 2005). Nagel et al. (1997) predicted the dose of BPA (fed to pregnant mice) that should be biologically active in mouse fetuses based on a comparison of BPA and estradiol in terms of both the relative affinity for nuclear ERs and binding to serum estrogen-binding proteins that effectively restrict estradiol (but not BPA) uptake into cells. This has been referred to as a "physiologic approach" to dose selection (vom Saal et al. 1998). Nagel et al. (1997) chose the fetal prostate growth bioassay to test the physiologically based prediction of low-dose estrogenic activity of BPA, although the prediction was that any estrogenic response would be altered by exposure to BPA during early development. Nagel et al. (1997) reported finding an enlarged prostate in male offspring after feeding pregnant mice 2 or 20 μg/kg/day BPA. Because these doses are below the current reference dose, this finding received a considerable amount of attention.

The findings by Nagel et al. (1997) raised a critical question: Why were the estrogenic effects that they observed below the current reference dose not predicted based on traditional toxicologic studies that focused on the toxic effects of very high doses of BPA (Morrissey et al. 1987)? The toxicologic approach involves dose selection based on the maximum tolerated dose, which can be described as "top-down dose selection," whereas the physiologic approach used by Nagel et al. (1997) can be described as "bottom-up dose selection" (Welshons et al. 2003). We show below that there is now overwhelming evidence demonstrating that these different experimental approaches lead to very different conclusions of safety with regard to the current reference dose for BPA of 50 μg/kg/day. Findings based on low-dose studies thus present a strong challenge to the assumptions that form the basis for chemical risk assessments.

Why Did the APC Contract with the HCRA to Write a Report on Low-Dose Effects of BPA?

The controversy created by reports of findings for BPA and other chemicals at "low doses," and studies funded by chemical corporations that quickly disputed these findings, resulted in the U.S. EPA asking the National Toxicology Program (NTP) to host a meeting in October 2000 on the low-dose issue. The final NTP Low Dose Peer Review report (NTP 2001) was summarized by the co-chairs and session organizers (Melnick et al. 2002).

In contrast to today, at the time of the NTP low-dose meeting there were relatively few published low-dose studies with BPA. However, the NTP report (NTP 2001) was critical of some of the industry-funded studies of BPA. For example, one industry-sponsored study (Ashby et al. 1999) was criticized by the NTP panel (NTP 2001, p. A9) for not identifying that the body weights and reproductive organ weights of the control animals were significantly different from those of control

animals used in the study that it was supposed to replicate (Nagel et al. 1997), and that the study was not a true replication because of the use of different animal feed. Another industry-funded study that concluded that all findings were not statistically significant (Elswick et al. 2000) was harshly criticized by the NTP panel as presenting conclusions that were "flawed," "illogical," and "misleading," and the NTP panel concluded that results were, in fact, statistically significant (NTP 2001, p. A89).

When the initial report of the NTP panel was released, the APC quickly issued a public letter in which the conclusion of the NTP panel—that there was "credible evidence of low-dose effects"—was described as "troubling . . . if not erroneous" (Bisphenol A Global Industry Group 2000). The APC then contracted with the HCRA in 2000, which established a panel of scientists (including coauthor C.H.) to perform a weight-of-the-evidence evaluation of available data on the developmental and reproductive effects of exposure to BPA in laboratory animals. In turn, the HCRA panel focused on 19 published studies of the available 47 publications and, particularly, on the effects of low doses of BPA on development of the reproductive system in male rodents. The conclusions in the panel's published report (Gray et al. 2004) were directed to this portion of the literature that was intensively scrutinized, but the wording was promptly interpreted by plastic industry trade organizations as suggesting that a far more complete survey of the BPA literature had been encompassed by the panel's review process (APM 2005; vom Saal 2005). As of April 2002, there were 47 available publications that could have been examined in a comprehensive review of all low-dose effects of BPA in all species. Because of the charge to the HCRA panel and its response to that charge, it reviewed 7 of 9 (78%) of the industry-funded published studies, but reviewed only 12 of 38 (38%) of the government-funded studies that were available in the published literature.

Current Status of Literature on Low-Dose Effects of BPA

Of a total of 115 published studies with low doses of BPA below the prior LOAEL of 50 mg/kg/day that we accessed via a PubMed search at the end of December 2004, there have been 94 published studies reporting *in vivo* estrogenic activity of BPA. Of the 94 low-dose studies reporting significant effects, 31 published studies have reported effects caused by doses of BPA at and below the reference dose of 50 µg/kg/day.

Rate of growth and sexual maturation, hormone levels in blood, reproductive organ function, fertility, immune function, enzyme activity, brain structure, brain chemistry, and behavior are all affected by exposure to low doses of BPA. Many of these effects are due to exposure during early development (gestation and/or lactation), but effects due to postweaning-through-adult exposure have also been reported.

A comprehensive review of the rapidly growing literature on adverse health effects of low doses of BPA in vertebrates and invertebrates is beyond the scope of this commentary, but references are available online (Endocrine Disruptors Group 2005). We describe below some examples of effects of low doses of BPA in mice and rats.

- Increased postnatal growth in both males and females occurred at maternal doses between 2.4 and 500 µg/kg/day (Honma et al. 2002; Howdeshell et al. 1999; Nikaido et al. 2004; Takai et al. 2000).
- Early onset of sexual maturation in females occurred at maternal doses between 2.4 and 500 µg/kg/day (Honma et al. 2002; Howdeshell et al. 1999; Nikaido et al. 2004).
- Altered plasma luteinizing hormone levels occurred at a maternal dose of 2 µg/kg/day (Akingbemi et al. 2004), and decreased plasma testosterone in males occurred at a maternal dose of 2 µg/kg/day (Akingbemi et al. 2004; Kawai et al. 2003).
- An increase in prostate size in male offspring occurred at maternal doses between 2 and 50 µg/kg/day (Gupta 2000; Nagel et al. 1997; Timms et al. 2005). A decrease in daily sperm production and fertility in males was also reported at doses between 0.2 and 20 µg/kg/day due to developmental or adult exposure (Al-Hiyasat et al. 2002; Chitra et al. 2003; Sakaue et al. 2001; vom Saal et al. 1998).
- Stimulation of mammary gland development in female offspring occurred at the very low maternal dose of 0.025 µg/kg/day delivered tonically by an Alzet pump (Markey et al. 2001a). Significant disruption of the alignment of chromosomes during meiosis was observed in developing oocytes during puberty because of leaching of BPA from polycarbonate drinking bottles at doses between 15 and 70 µg/kg/day (Hunt et al. 2003), and an increase in mortality of embryos occurred at a maternal dose of 25 µg/kg/day (Al-Hiyasat et al. 2004). Disruption of adult estrous cycles occurred at maternal doses between 100 and 500 µg/kg/day (Nikaido et al. 2004; Talsness et al. 2000).
- Altered immune function occurred at doses between 2.5 and 30 µg/kg/day (Sawai et al. 2003; Yoshino et al. 2003, 2004).
- A decrease in antioxidant enzymes occurred at the very low dose of 0.2 µg/kg/day in adult males (Chitra et al. 2003).
- Changes in the brain include an increase in progesterone receptor mRNA levels at 400 µg/kg/day (Funabashi et al. 2003), ER-α levels at 40 µg/kg/day (Aloisi et al. 2001), and ER-β mRNA levels at 25 µg/kg/day (Ramos et al. 2003) and a change in brain somatostatin receptors at 400 µg/kg/day (Facciolo et al. 2002).
- Behavioral effects include hyperactivity at 30 µg/kg/day (Ishido et al. 2004), an increase in aggressiveness at 2–40 µg/kg/day (Farabollini et al. 2002; Kawai et al. 2003), altered reactivity to painful or fear-provoking stimuli at 40 µg/kg/day (Aloisi et al. 2002), and impaired learning at 100 µg/kg/day (Negishi et al. 2004). Developmental exposure to BPA also resulted in a significant change in the locus coeruleus, where BPA at 30 µg/kg/day reversed the normal sex differences in this brain structure and eliminated sex differences in behavior (Kubo et al. 2003). Developmental exposure decreased maternal behavior at 10 µg/kg/day (Palanza et al. 2002), altered play and other sociosexual behaviors at 40 µg/kg/day (Aloisi et al. 2002; Dessi-Fulgheri et al. 2002), and enhanced the behavioral response to drugs such as amphetamine at 40–300 µg/kg/day (Adriani et al. 2003; Suzuki et al. 2003).

Factors Accounting for the Absence of Significant Effects in Low-Dose BPA Experiments

As of the end of 2004, we are aware of 21 studies that report no harm in response to low doses of BPA. Source of funding is highly correlated with positive or negative findings in published articles. For government-funded published studies, 94 of 104 (90%) report significant effects at doses of BPA < 50 mg/kg/day. No industry-funded studies (0 of 11, or 0%) report significant effects at these same doses (Table 1). It is thus reasonable to pose two questions: *a*) Are government-funded scientists under real or perceived pressure to find or publish only data suggesting adverse outcomes? *b*) Are industry-funded scientists under real or perceived pressure to find or publish only data suggesting negative outcomes?

It is important to determine what specific factors, other than just source of funding, are associated with reports of no significant effects of low doses of BPA. In this article we discuss four issues, some of which have became apparent because of findings published after the cut-off date for the literature review in the HCRA report: strain of experimental animal, misinterpretation of finding no significant effects for the positive controls, animal feed, and specific end point examined.

Strain of experimental animal. The importance of the strain of animal used in low-dose BPA research was acknowledged in the HCRA report (Gray et al. 2004) as well as the previous NTP report (NTP 2001). The NTP panel emphasized the need to test for the sensitivity

of any animal model by including a positive control, such as the well-characterized estrogenic drugs DES and ethinylestradiol, and stated that

> Because of clear species and strain differences in sensitivity, animal model selection should be based on responsiveness to endocrine active agents of concern (i.e., responsive to positive controls), not on convenience and familiarity. (NTP 2001, p. vii)

A recent study has revealed the very low sensitivity to any estrogen of the Charles-River Sprague-Dawley (CD-SD) rat used in two studies (Ema et al. 2001; Tyl et al. 2002) that were heavily relied on by the HCRA panel in drawing the conclusion "that the negative findings for reproductive endpoints for rats are more compelling than the positive findings" (Gray et al. 2004). According to Charles River Laboratories (2004), rats were purchased by Charles River from Sprague-Dawley in 1950. This colony was continuously subjected to selective breeding for rapid postnatal growth and large litter size, and then in both 1991 and 1997 new colonies were established from selected animals.

Yamasaki et al. (2002) reported that the CD-SD strain of rat showed some responses to 50-μg/kg/day ethinylestradiol administered for 28 days, and more responses to the very high dose of 200 μg/kg/day. Ethinylestradiol is the potent estrogenic drug used by women in birth control pills at a dose of 0.5 μg/kg/day (based on a body weight of 60 kg). The CD-SD rat thus has a very low sensitivity to ethinylestradiol, because relative to women, it requires 100- to 400-fold higher doses to produce effects. In contrast, the fetal male CF-1 mouse examined in the initial vom Saal laboratory studies with BPA responded to ethinylestradiol with significant changes in adult sperm production and prostate size at a maternal oral dose of 0.002 μg/kg/day (Thayer et al. 2001). The CF-1 male mouse fetus is thus between 25,000 and 100,000 times more sensitive to ethinylestradiol relative to the CD-SD rat. Yamasaki et al. (2002) also reported that 600 mg/kg/day BPA was required to see effects in CD-SD rats. This dose is > 200,000 times higher than the BPA doses used in studies conducted in the vom Saal laboratory (Howdeshell et al. 1999, Nagel et al. 1997), and as indicated above, it is also dramatically higher than doses of BPA required to cause effects in > 90 other low-dose BPA studies conducted with other types of rats, various mouse strains, and other experimental vertebrate and invertebrate animals. There are now many studies that have been conducted with rats other than the CD-SD strain that show low-dose effects of BPA, but very few of these studies were subject to review by the HCRA panel (Gray et al. 2004).

All studies with CD-SD rats report the absence of significant effects of low doses of BPA (Table 1), although the conclusions in one these studies (Elswick et al. 2000) were questioned by the NTP panel (NTP 2001). If the studies that used the CD-SD rat are eliminated from consideration, 94 of 98 (96%) government-funded studies report significant effects of low doses of BPA, whereas 0 of 8 (0%) industry-funded studies reports significant effects with the same low doses (Table 1).

Misinterpretation of the absence of significant findings for the positive controls. The very low sensitivity of the CD-SD rat strain to BPA was predicted by its low sensitivity to ethinylestradiol when it was included as a positive control. Two industry-funded studies (Ashby et al. 1999; Cagen et al. 1999) were designed with DES included as a positive control, which was reported by industry spokesmen (Toloken 1998) at a public news briefing about the Cagen et al. (1999) study. A critique [Environmental Data Services (ENDS) 1998] pointed out that the positive control, DES, failed to show a difference from the negative controls in each of these studies (Ashby et al. 1999; Cagen et al. 1999); however, the authors did not indicate in their published articles that DES had been used as the positive control. Subsequent studies funded by chemical corporations, all of which have reported the absence of significant effects for low doses of BPA, avoided this problem by simply not including a positive control in the experiment.

The NTP panel (NTP 2001) commented on the issue regarding

> . . . a study in which the positive control does not produce the expected positive response. The prudent course of action in such cases may be to declare the study inadequate and repeat it, regardless of the experimental outcome in the test groups. (NTP 2001, pp. 5–10)

The NTP panel went on to note that,

> For those studies that included DES exposure groups, those that showed an effect with BPA showed a similar low-dose effect with DES (e.g., prostate and uterus enlargement in mice), while those that showed no effect with BPA also found no effect with DES.

As articulated by the NTP panel (NTP 2001), only by including a known estrogenic chemical, such as DES or ethinylestradiol, as a positive control in an experiment can the reason for the failure to find low-dose estrogenic effects of BPA be determined to be due to either inactivity of the chemical, insensitivity of the model animal, or some other variable, such as the type of feed used.

Disruption of low-dose studies of endocrine-disrupting chemicals by variability in components of commercial animal feed. A critical issue in experiments concerning effects of low doses of estrogenic chemicals is that a common rodent feed used in toxicologic studies has been reported by investigators at the National Institute of Environmental Health Sciences (Thigpen et al. 2003) to be highly variable in its estrogenic activity. These investigators reported that some batches of this feed were able to interfere with the ability to detect puberty-accelerating effects of DES in female CD-1 mice, due to the feed maximally advancing the age at puberty in control females (Thigpen et al. 2003). The use of this particular feed by Cagen et al. (1999) and Tyl et al. (2002) raises the possibility that endocrine-disrupting components in this feed played a role in the failure of these studies to show low-dose effects of BPA; Cagen et al. (1999) also failed to find significant effects of the positive control DES, whereas Tyl et al. (2002) did not include a positive control. The HCRA panel (Gray et al. 2004) relied heavily on both of these studies.

Both the NTP (2001) and HCRA panels (Gray et al. 2004) raised the possibility that the type of feed used in some studies may have affected the results, but the information provided by Thigpen et al. (2003) about variability in estrogenic activity in different batches of a feed commonly used in toxicologic studies, and other recent findings regarding variability in endocrine-disrupting components of feed other than phytoestrogens (vom Saal et al. 2004, 2005), was not available for either the NTP or the HCRA panel to review. Thus, it is understandable that the HCRA report could state: "Nor is there compelling evidence that the type of feed administered . . . can explain the negative results reported" (Gray et al. 2004).

It is now clear that it is necessary to develop a standard feed that is appropriate for studies involving the examination of end points that are sensitive to estrogenic chemicals, because estrogenic as well as other components of feed can be present in highly variable amounts in different batches; levels of phytoestrogens in plants vary in response to different environmental conditions. The findings reported by Thigpen et al. (2003) that effects of DES could be masked by some batches of a commercial feed clearly demonstrate that, without an

Table 1. Biased outcome due to source of funding in low-dose *in vivo* BPA research as of December 2004.

	All studies		CD-SD rat studies		All studies except CD-SD rats	
Source of funding	Harm	No harm	Harm	No harm	Harm	No harm
Government	94 (90.4)	10 (9.6)	0 (0%)	6 (100)	94 (96)	4 (4)
Chemical corporations	0 (0)	11 (100)	0 (0%)	3 (100)	0 (0)	8 (100)

Values shown are no. (%).

appropriate positive control, false-negative findings can occur that lead to the false conclusion that even biologically active doses of potent estrogenic drugs such as DES have no effect.

The uterotrophic response is not stimulated by low doses of BPA. Seven articles have reported that low doses of BPA do not stimulate a uterotrophic response (Ashby and Odum 2004; Diel et al. 2004; Gould et al. 1998; Laws et al. 2000; Markey et al. 2001b; Mehmood et al. 2000; Tinwell et al. 2000). For example, a dose of 100 mg/kg/day BPA injected subcutaneously was required to stimulate an increase in uterine weight in prepubertal CD-1 mice (Markey et al. 2001b). This is in marked contrast to the fetal CD-1 mouse prostate (Gupta 2000; Timms et al. 2005), testes (Kawai et al. 2003), mammary glands (Markey et al. 2001a), and brain (Palanza et al. 2002), which all respond to doses of BPA at and far below the reference dose of 50 μg/kg/day. In order to assess the effects of low doses of BPA or other estrogenic endocrine-disrupting chemicals on the uterus, more sophisticated approaches are required than just measuring uterine weight (Markey et al. 2001b; Newbold et al. 2004).

Implications for Risk Assessments of Low-Dose BPA Effects

As noted above, BPA is a widely used chemical, with a capacity in excess of 6.4 billion lb in 2003. If regulatory agencies were to determine that the actual LOAEL for BPA is below the current reference dose of 50 μg/kg/day, the 15 corporations that manufacture BPA would be affected economically (Burridge 2003). However, corporations that manufacture products made from BPA would be less affected because alternatives to BPA already exist for many products. Potential economic impacts need to be considered in relation to the implications for human health of the wide range of adverse effects caused by exposure to very low doses of BPA in the animal experiments described above.

Measurements of current human contamination indicate that exposure of human fetuses to BPA already occurs at levels within the range demonstrated to cause adverse effects in fetal rodents (Schonfelder et al. 2002). Specifically, Zalko et al. (2002) injected pregnant CD-1 mice subcutaneously on gestation day 17 with 25 μg/kg tritiated BPA; parent (unconjugated) BPA levels in mouse fetuses at 0.5, 2, and 24 hr after administration were 4.20, 0.48, and 0.13 ng/g (ppb), respectively. Schonfelder et al. (2002) reported that parent BPA levels in human fetal serum ranged from 0.2 to 9.2 ng/mL (ppb), and the median was 2.3 ng/mL (ppb). Many adverse effects have been reported in offspring due to maternal doses of ≤ 25 μg/kg/day in mice.

That the adverse effects being observed at low doses of BPA in animal experiments should be of concern with regard to human health is shown by a study comparing BPA levels in nonobese and obese women in Japan who had normal ovarian function or polycystic ovarian disease. In this case–control study Takeuchi et al. (2004) reported significantly higher blood levels of BPA both in obese women and in women with polycystic ovarian disease. These findings suggest that the adverse effects due to exposure to low doses of BPA in experimental animals may be predictive of adverse effects in adult humans.

The implications of these results extend beyond BPA, because they may lead to requirements that hazard assessments be designed to detect analogous low-dose impacts of other chemicals. Acknowledgment of the existence of the large number of studies showing unique low-dose effects of BPA could lead to the demand that, in designing studies to assess the hazards of all chemicals for risk assessment purposes, a wider range of doses must be examined, as opposed to only a few very high doses based on the maximum tolerated dose. This would require accepting that extrapolation from data on effects at very high doses (based on the linear-threshold model) is not valid for endocrine-disrupting chemicals (vom Saal and Sheehan 1998; Welshons et al. 2003).

We posed above the question concerning why a large number of estrogenic effects have been observed in studies that examined low doses of BPA, but these effects were not predicted based on traditional toxicologic studies that focused on the toxic effects of very high doses of BPA. When evidence of a nonmonotonic, inverted-U dose–response relationship is found in a toxicologic study, the results are often identified as not showing a dose–response relationship. Although findings in toxicologic studies that occur within a low-dose range but not at higher doses are typically discounted or, at best, considered to be rare, just for BPA there are currently 11 *in vivo* and *in vitro* examples of unique effects seen at low doses but not at higher doses (e.g., Endocrine Disruptors Group 2005; Oehlmann et al. 2000; Wetherill et al. 2002; Welshons et al. 2003; Wozniak et al. 2005).

The inverted-U dose–response phenomenon shows that dose selection is critical in studies of chemicals such as BPA, and older toxicologic studies that just examined a few very high doses are not relevant for assessing the possibility of unique effects that only occur within a specific low-dose range. The mechanisms mediating qualitative changes in response over a wide range of doses are now being elucidated at multiple levels, such as gene-response profile (Coser et al. 2003), changes in tissue expression of receptors (Gupta 2000), and changes in neuroendocrine feedback systems (Rubin et al. 2001; Talsness et al. 2000). Low doses of a hormone can stimulate a response, whereas much-higher doses inhibit the same response, and this phenomenon is so well established that it is used in clinical endocrinology to treat diseases (Kappy et al. 1989; Welshons et al. 2003). Although endocrinologists find it plausible that there are unique effects caused by low doses of a chemical with hormonal activity that might not be observed at much higher doses, this has not been recognized by regulatory agencies involved in risk assessments.

It is important to keep in mind that traditional toxicologic testing of chemicals for regulatory purposes requires examination of only a few very high doses of a chemical, which often do not exceed 50-fold below the maximum tolerated dose (vom Saal and Sheehan 1998). The maximum tolerated dose of BPA is very high (~ 1,250,000 μg/kg/day; Morrissey et al. 1987; IRIS 1988). In contrast, a wide range of adverse effects in 31 published studies have been reported in offspring due to administering pregnant mice and rats doses of BPA 25,000 times lower than the maximum tolerated dose (Endocrine Disruptors Group 2005). The conclusion from these published findings is that examining only a 50-fold dose range on the basis of the maximum tolerated dose is a seriously flawed approach for assessing adverse effects of chemicals that are mediated by highly sensitive endocrine-response mechanisms.

Regulatory agencies readily accept that the predicted reference dose is actually "safe" without ever requiring that this dose be verified in an experiment to cause no adverse effects (vom Saal and Sheehan 1998; Welshons et al. 2003). Regulatory agencies need to acknowledge that there is now overwhelming evidence for adverse effects of one of the highest-volume chemicals in commerce below the previously predicted "safe" daily dose for humans. This should lead to the requirement that new risk assessments be conducted to reevaluate the safety of other chemicals, in addition to BPA.

Conclusions and Recommendations

In summary, a comprehensive up-to-date analysis by regulatory agencies is needed to evaluate the potential hazards to humans from exposure to BPA at doses below the prior LOAEL of 50 mg/kg/day; low doses of BPA have now been reported to alter brain chemistry and structure, behavior, the immune system, enzyme activity, the male reproductive system, and the female reproductive system in a variety of animals, including snails, fish, frogs, and mammals. There are also a number of *in vitro* studies showing that the particular type of ER (α or β) and the specific coregulators present in cells can markedly influence the dose of BPA required to stimulate a response

(e.g., Routledge et al. 2000). This is consistent with estrogen-responsive tissues within the same animal showing marked differences in the dose of BPA required to elicit a response.

Not all effects of BPA are mediated by the classical nuclear ERs (α and β). Very low part-per-trillion doses of BPA can stimulate responses in cultured mouse pancreas cells, rat pituitary tumor cells, and human breast cancer cells via rapid induction of calcium uptake (Quesada et al. 2002; Walsh et al. 2005; Wozniak et al. 2005); these same low doses of BPA stimulate proliferation in mouse (Gupta 2000) and human (Wetherill et al. 2002) prostate cells in culture. Nongenomic cell signaling systems involve serial activation of kinases via ligand binding to receptors associated with the cell membrane, and these pathways are known to have tremendous amplifying capacity.

In the now outdated perspective of the HCRA report (Gray et al. 2004), it was stated that "In the case of BPA the only proposed mechanism for low-dose effects is through modulation of the [nuclear] estrogen receptor." Instead, the recent findings concerning the multiple mechanisms of action of BPA show that at concentrations < 1 ppt, BPA activates receptors associated with the plasma membrane of selected target cells. As the BPA "dose at target" increases, various responses in the same or different cells are activated or inhibited (MacLusky et al. 2005), with the specific dose required being dependent on the subtype of nuclear ER and specific coactivators or coinhibitors that are present. At even higher concentrations (parts per billion to parts per million), inhibition of androgen-stimulated and thyroid-hormone–stimulated responses can also occur. That the integrated output across a 1-million-fold dose range can be nonmonotonic (inverted-U shape) is thus not unexpected by scientists who study hormones and hormonally active drugs or chemicals (Welshons et al. 2003). Regulatory agencies that conduct risk assessments have not addressed the implications of nonmonotonic dose–response curves for endocrine-disrupting chemicals with regard to the linear-threshold model currently used to predict "safe" doses for humans.

The *in vitro* findings at low (and even sub) part-per-trillion doses of BPA have to be viewed in relation to potential effects of free (unconjugated and unbound) BPA levels in human blood. Data from numerous studies show that unconjugated BPA in human blood and tissues is in the low part-per-billion range (Endocrine Disruptors Group 2005) and that BPA shows limited binding to human plasma binding proteins that regulate the uptake of estrogenic chemicals into tissues (Nagel et al. 1999). Importantly, new analytical methods have been developed since the published literature was reviewed in 1998 for the last BPA risk assessment conducted by the European Union (ECB 2003). These new methods have now made it possible to detect BPA in blood within the range that it shows biologic activity, which was not previously the case. There is thus convincing evidence that biologically active levels of BPA in human blood are above the range that has been demonstrated to cause changes in function in human tissues based on *in vitro* studies.

The literature we reviewed shows that the rate of leaching from commonly used BPA-containing products (the lining of tin cans and polycarbonate food and beverage containers) is high enough to result in adverse effects in laboratory animals (Raloff 1999). These recently published findings indicate that the accepted migration limit [recently set by the European Union (ECB 2003)] of 30 ppb BPA from polycarbonate or resins into food and beverages is not sufficiently protective of human health. The case–control study reporting that ovarian disease in Japanese women is related to blood levels of BPA provides a first confirmation of this prediction in adult humans (Takeuchi et al. 2004).

Almost one-half of the low-dose BPA studies have been published in just the last 2 years, and there were only five published low-dose BPA studies as of 1998 when the initial comprehensive literature search was conducted for the last risk assessment conducted by the European Union (ECB 2003). A thorough analysis of the entire published low-dose BPA literature associated with a new risk assessment by regulatory agencies that takes into account the issues discussed here and elsewhere is now warranted (vom Saal 2005; vom Saal and Sheehan 1998; vom Saal et al. 2004, 2005, in press; Welshons et al. 2003). It is important that a reexamination of the BPA low-dose literature include a discussion of the requirement for appropriate positive controls, which was identified by the NTP panel as a significant problem in studies claiming to find no low-dose effects.

The initial NTP review concerning BPA as a carcinogen concluded that "there was no convincing evidence that [BPA] was carcinogenic," because the background level of cancer was high in untreated males (NTP 1982). This conclusion has been criticized by a scientist in the NTP: Huff (2002) concluded that, if these findings (NTP 1982) were officially reanalyzed based on the approach to interpreting cancer in animal studies used today, BPA would be interpreted as being associated with an increase in tumors of blood cells, the testes, and the mammary glands.

In summary, a new risk assessment is needed to establish a new LOAEL and a new reference dose for BPA based on the extensive new information from low-dose studies. In addition, the low-dose literature for BPA and other endocrine-disrupting chemicals shows that regulatory agencies need to begin the process of reevaluating the assumptions that provide the basis for the linear-threshold model used in risk assessments.

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Exhibit D

Current Biology, Vol. 13, 546–553, April 1, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/S0960-9822(03)00189-1

Bisphenol A Exposure Causes Meiotic Aneuploidy in the Female Mouse

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Summary

Background: There is increasing concern that exposure to man-made substances that mimic endogenous hormones may adversely affect mammalian reproduction. Although a variety of reproductive complications have been ascribed to compounds with androgenic or estrogenic properties, little attention has been directed at the potential consequences of such exposures to the genetic quality of the gamete.

Results: A sudden, spontaneous increase in meiotic disturbances, including aneuploidy, in studies of oocytes from control female mice in our laboratory coincided with the accidental exposure of our animals to an environmental source of bisphenol A (BPA). BPA is an estrogenic compound widely used in the production of polycarbonate plastics and epoxy resins. We identified damaged caging material as the source of the exposure, as we were able to recapitulate the meiotic abnormalities by intentionally damaging cages and water bottles. In subsequent studies of female mice, we administered daily oral doses of BPA to directly test the hypothesis that low levels of BPA disrupt female meiosis. Our results demonstrated that the meiotic effects were dose dependent and could be induced by environmentally relevant doses of BPA.

Conclusions: Both the initial inadvertent exposure and subsequent experimental studies suggest that BPA is a potent meiotic aneugen. Specifically, in the female mouse, short-term, low-dose exposure during the final stages of oocyte growth is sufficient to elicit detectable meiotic effects. These results provide the first unequivocal link between mammalian meiotic aneuploidy and an accidental environmental exposure and suggest that the oocyte and its meiotic spindle will provide a sensitive assay system for the study of reproductive toxins.

Introduction

An estimated 10%–25% of fertilized human oocytes are aneuploid; thus, numerical chromosome abnormalities are the leading cause of miscarriage, congenital defects, and mental retardation [1]. Because almost all such aneuploidy derives from meiotic errors, considerable effort has been directed at identifying factors that increase meiotic nondisjunction. A number of potential risk factors, including irradiation (e.g., [2, 3]), smoking or drinking (e.g., [4, 5]), oral contraceptives and fertility drugs (e.g., [4, 6]), and environmental pollutants/pesticides (e.g., [7]), have been suggested. However, significant effects have been small and difficult to verify or disputed, making positive associations hard to establish. In part, this may reflect difficulties in detection. For example, the extraordinary effect of maternal age on aneuploidy may obscure less obvious associations. Further, previous studies may have focused on the "wrong" population; that is, most utilized liveborns, although virtually all aneuploidy terminates in miscarriage. Thus, the contribution of environmental insults to meiotic chromosome errors remains unknown.

We recently experienced an inadvertent environmental exposure in our mouse colony to 2,2-(4,4-dihydroxydiphenol)propane, or bisphenol A. Bisphenol A (BPA) is the monomer that is polymerized to manufacture polycarbonate plastic products and resins, such as those used to line cans containing food and beverages and those found in dental sealents. The exposure was accompanied by highly significant increases in meiotic chromosome abnormalities, including nondisjunction; thus, bisphenol A was implicated as a potent disruptor of meiosis. The ability to experimentally recreate the exposure has allowed us to verify our initial observations and conduct dose-response studies.

Results

A Sudden Increase in Meiotic Abnormalities Is Correlated with Damage to Caging Materials

We recently reported meiotic studies of mouse mutants with defects in the alignment of the chromosomes on the first meiotic (MI) spindle [8]. This meiotic abnormality, which we have termed congression failure (Figure 1), is of particular relevance to humans because it is an age-related feature of human oocytes and has been postulated to be causally related to the well-known increase in aneuploidy associated with advancing maternal age [9].

In the course of meiotic studies of mouse oocytes conducted in 1998, we observed a sudden and dramatic change in congression failure levels. The first wave of follicles that initiate growth in the sexually immature ovary provides access to a large cohort of oocytes, and, typically, only 1%–2% of oocytes from control females exhibit congression failure at metaphase I [8]. However, in experiments conducted in August 1998, congression failure levels suddenly spiked, and approximately 40% of control oocytes exhibited this phenotype or more severe aberrations (Figures 1 and 2).

At the same time that these studies were being conducted, we were also using the animal facility to house

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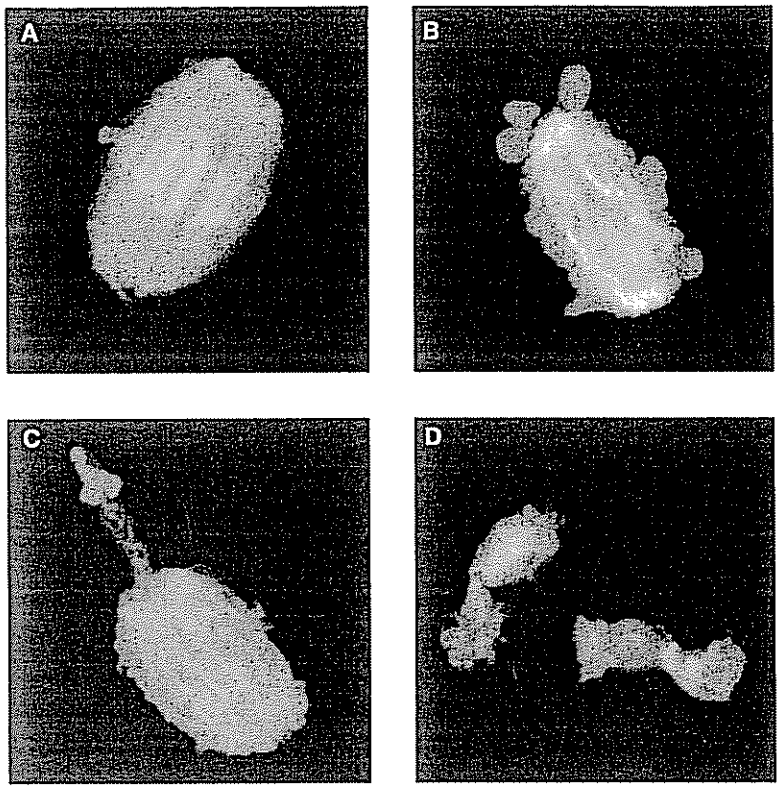

Figure 1. Normal and Abnormal Meiotic Metaphase Configurations

Confocal images of intact mouse oocytes immunostained with an antibody to β-tublin to visualize the meiotic spindle (green) and counterstained with propidium iodide to visualize the chromosomes (red).

(A) Normal metaphase I configuration.

(B–D) Representative meiotic abnormalities from exposed females. (B) Congression failure in an MII-arrested oocyte. Most abnormalities were of this type, but, at the time of maximal exposure, others were observed (e.g., [C] metaphase I cell with chromosomes that have been ejected from the spindle; [D] a cell that should be undergoing the first meiotic division but appears to have two separate groups of chromosomes in a telophase-like configuration).

mice for another analysis of meiotic chromosome segregation. This second set of studies involved cytogenetic analyses of meiosis II preparations in paracentric inversion-carrying female mice and controls and was designed to ask whether meiotic nondisjunction might be increased in inversion heterozygotes. In total, we analyzed five different inversions involving either chromosome 2, 19, or the X chromosome (In(2)2H, In(2)5Rk, In(2)40Rk, In(19)37Rk, and In(X)1H) [10]. However, in the context of the present report, the data on inversion heterozygotes were unhelpful, since data were not collected at all relevant exposure time points; thus, the following discussion pertains only to control animals.

Data on aneuploidy levels were collected for three different types of control animals: inversion homozygotes, chromosomally normal sibs of inversion heterozygotes, and unrelated chromosomally normal (C57BL/6) animals. A dramatic increase in aneuploidy levels in these animals coincided with the increase in congression failure levels. Meiotic aneuploidy is infrequent in the mouse, and baseline rates of hyperploidy (i.e., cells with >20 chromosomes, representing one-half of all nondisjunctional events) are approximately 0.5%–1.0% [11]. Our initial studies were consistent with this expectation; the level of hyperploidy in 415 metaphase II control oocytes was 0.7%. However, beginning in August of 1998 and continuing for several months, analyses of control oocytes indicated an extraordinary increase in aneuploidy (Figure 3; Table 1). Overall, 20/345 (5.8%) oocytes were hyperploid, a highly significant increase ($\chi^2 = 16.53$, $p < 0.001$).

Thus, two independent meiotic studies indicated surprising increases in abnormalities at exactly the same time. Further, the concomitant increases in congression

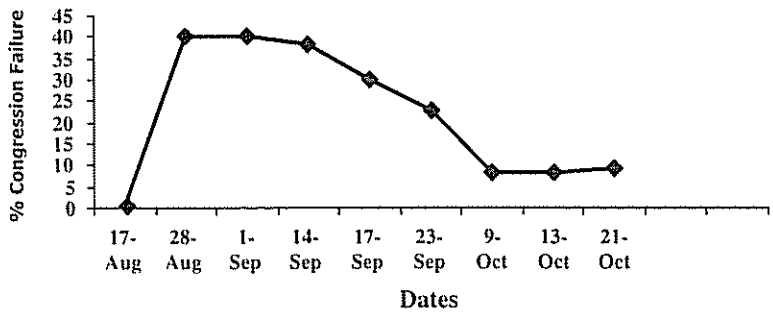

Figure 2. Levels of Congression Failure at Metaphase I in Control Oocytes before and during the Exposure

Our previous studies have established a consistent level of 1%–2% congression failure at metaphase I in ooyctes from control females [8]. Experiments run 2 weeks apart in August 1998 revealed a highly significant increase ($p < 0.001$) in congression failure in oocytes from control females on the C57BL/6 background. In subsequent weekly experiments, values remained high until the end of September (all time points were significantly higher than expectation at $p < 0.01$), when the most visibly damaged cages were replaced by new polycarbonate cages. While the level of congression failure declined to approximately 10% during October, all values remained significantly higher than expected ($p < 0.05$). Similar results were obtained for females on the C3H inbred background (data not shown).

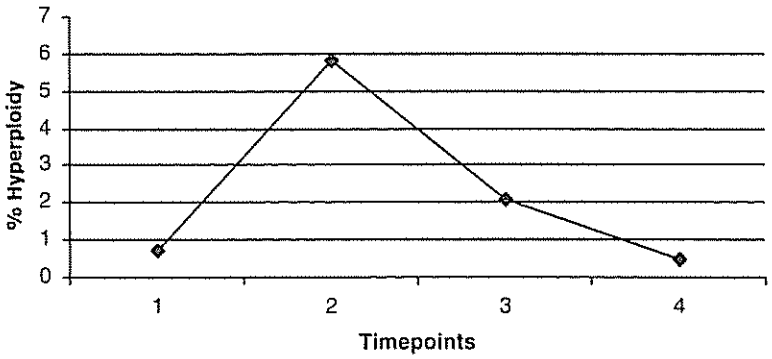

Figure 3. Levels of Hyperploidy in Oocytes before, during, and following Exposure

Levels of hyperploidy (one half of all nondisjunction events) in control oocytes before (1/98–7/98), during (8/98–4/99), and after (5/99–12/00) the exposure (time points 1, 2, and, 3, respectively) and following transfer of the mouse colony to a "clean" facility (time point 4). The numbers of control oocytes analyzed during time points 1, 2, 3, and 4 were 415, 345, 959, and 204, respectively.

failure and aneuploidy were consistent with earlier studies suggesting that congression failure at metaphase I is often "translated" into nondisjunction [8].

Subsequent studies allowed us to rule out an effect of medium constituents or culture conditions as the source of the meiotic disturbances and allowed us to determine that the changes coincided with damage to caging materials caused by the inadvertent use of a harsh alkaline detergent, A33 (Airkem Professional Products, Ecolab; see [12] for further details). Both cages and water bottles were comprised of polycarbonate plastic. A monomer used in the production of polycarbonate, bisphenol A (BPA), is weakly estrogenic in some assay systems (e.g., uterus [13, 14] and human MCF-7 breast cancer cells [15]) and is known to leach from polycarbonate [16]; thus, it seemed possible that the meiotic disturbances were the result of BPA exposure.

Cause and Effect: Confirmation that the Meiotic Effects Are Mediated by Caging Materials

As the most severely damaged cages were pulled from use, a corresponding drop in the level of congression failure was observed (Figure 2), providing indirect evidence that the effect was mediated by the damaged caging materials. Similarly, a third round of cytogenetic studies conducted after the elimination of all damaged caging from the animal facility (May 1999–November 2000) demonstrated a decrease in hyperploidy levels to 2.2% (Figure 3). Because this value was still 3-fold higher than values obtained prior to the exposure, raising the possibility of a residual effect, the colony was moved to a separate housing facility outfitted with new polysulfone cages and glass water bottles, and new breeding stock was purchased. Nondisjunction studies of control females born and raised in the facility confirmed a return to pre-exposure levels of hyperploidy (Figure 3), and the level of congression failure in several hundred oocytes analyzed from offspring of different mating cages demonstrated a return to typical control levels (i.e., 1%–2%; data not shown).

To assess directly the link between damaged polycarbonate caging materials and meiotic disruption, a series of studies involving the assessment of chromosome alignment in metaphase II (MII)-arrested oocytes was initiated. This stage was chosen for two reasons. First,

Table 1. Incidence of Hyperploidy in MII-Arrested Oocytes of Control Animals Analyzed before, during, or after the Environmental Exposure, or in a Separate "Clean" Animal Facility

Time Interval	Strain/Genotype	Age	Number of Cells	Number of (%) Hyperploid
BEFORE (prior to August 1998)	In(2)2H/In(2)2H	4 weeks	415	3 (0.7)
DURING (September 1998–April 1999)	In(2)2H/In(2)2H	4 weeks	104	5 (4.8)
	In(19)37Rk/In(19)37Rk	4 weeks	89	7 (7.9)
	In(2)5Rk (+/+)[a]	4 weeks	29	1 (3.4)
	C57BL/6	8–12 months	32	3 (9.4)
	In(2)2H/In(2)2H	8–12 months	91	4 (4.4)
	Pooled		345	20 (5.8)
AFTER (May 1999–December 2000)	In(19)37Rk/In(19)37Rk	4 weeks	183	5 (2.7)
	In(X)1H/In(X)1H	4 weeks	133	2 (1.5)
	In(2)40Rk/In(2)40Rk	4 weeks	73	2 (2.7)
	C57BL/6	4 weeks	425	12 (2.8)
	In(2)5Rk (+/+)[a]	4 weeks	145	0 (0)
	Pooled		959	21 (2.2)
NEW FACILITY (December 2000–February 2001)	C57BL/6	4 weeks	204	1 (0.5)

Control animals consisted of inversion homozygotes, chromosomally normal sibs of inversion heterozygotes, and unrelated chromosomally normal (C57BL/6) animals.

[a]The inversion In(2)5Rk is homozygous lethal. Stock matings thus generated both inversion heterozygotes (In(2)5Rk/+) and normal sequence (+/+) progeny.

Table 2. Meiotic Effects Mediated by Caging Materials

	Total MII-Arrested Oocytes	Congression Failure	χ^2 Value
Control: New Cages/Glass Bottles	271	5 (1.8%)	
Damaged Cages/Glass Bottles			
Mild	401	35 (8.7%)	13.62, $p < 0.001$
Severe	149	30 (20.1%)	42.20, $p < 0.001$
Damaged Bottles	197	53 (26.9%)	66.05, $p < 0.001$
Damaged Cages/Damaged Bottles	58	24 (41.4%)	93.19, $p < 0.001$
Damaged Bottles (Conventional Polycarbonate)	134	40 (29.9%)	71.12, $p < 0.001$

oocytes that complete the first division remain arrested at MII until fertilization; thus, this time point provides access to a nontransient metaphase stage. Second, the analysis of chromosome alignment is both more rapid and more cost effective than traditional cytogenetic analysis of aneuploidy. New high-temperature polycarbonate (polyphthalate carbonate) cages were exposed to a single contact with dilute (1:64) or full strength A-33 detergent. Control animals were housed in new polysulfone cages, and glass water bottles were used for both treated and control cages. Analysis of MII-arrested oocytes from normal females born and raised in intentionally damaged cages revealed a direct correlation between the degree of damage and the level of meiotic disturbance, and offspring from mildly and severely damaged cages exhibited an ~5-fold and >10-fold increase in congression failure, respectively (Table 2). Although both mildly and severely damaged cages exhibited significant increases compared to controls (Table 2), the level of congression failure did not approach that observed during the initial incident. This finding suggests that water bottles were an additional source of exposure. Indeed, subsequent analyses of damaged bottles revealed significant increases in congression failure (Table 2), and experiments involving both intentionally damaged cages and bottles also resulted in a significant increase (Table 2) and produced levels comparable to those at the height of the original exposure.

The high-temperature, autoclavable polycarbonate cages and bottles used in our facility were comprised of a synthetic thermoplastic polymer that is a blend of bisphenol A and polyester carbonate. Thus, it remained possible that the meiotic effects were not mediated by BPA. To test this, we conducted a series of experiments with conventional polycarbonate bottles (i.e., comprised only of BPA) that were intentionally damaged by exposure to dilute (1:64) A-33 detergent and subsequently washed in water but not autoclaved. As shown in Table 2, the congression failure rate for this type of damaged bottle was highly significantly increased over controls and was virtually identical to the level observed for the high-temperature polymer. This set of experiments suggests that, following chemical damage, BPA continues to leach from polycarbonate even in the absence of further harsh treatment (e.g., autoclaving).

Thus, meiotic analyses both at the time of the initial exposure and in subsequent studies using intentionally damaged caging materials provided strong circumstantial evidence of a link between BPA exposure and the meiotic disturbances.

BPA Exposure: Determining the Dosage and Timing Necessary to Induce Meiotic Effects

To characterize the timing and exposure levels necessary to induce meiotic defects, a series of studies involving the treatment of females with daily oral doses of BPA was initiated. As a first step, we estimated the constant exposure levels that elicited a demonstrable effect in the water bottle experiments (Table 2) by determining BPA levels in water from damaged bottles. Gas chromatography-mass spectrometry analysis with electron impact ionization methodology indicated approximate BPA levels of 100 and 360 ng/ml (Figure 4). Assuming an oral intake of 4–5 ml water per day and an average weight of 25–28 g/mouse, daily exposure levels from water were estimated to be 14–72 ng/g body weight. Using these doses as a guideline, we directly tested the meiotic effects of BPA by treating juvenile females (20- to 22-day-old mice) with 20, 40, or 100 ng/g body weight/day oral doses of BPA for 6–8 days preceding oocyte analysis. Low oral doses of BPA have been suggested to be subject to rapid first-pass elimination by the liver and efficient metabolic clearance [17, 18]. Nevertheless, a significant increase in congression failure (Table 3; [$\chi^2 = 4.79$; $p < 0.05$]), and a dose-related increase in the level of abnormalities, was observed among the treated animals. Thus, it appears that low-dose BPA exposure during the final stages of follicle growth is sufficient to cause meiotic abnormalities. To determine the shortest exposure that produced detectable effects, an additional set of experiments using a dose of 20 ng/g for 3, 5, or 7 days prior to oocyte analysis was conducted. All three exposures resulted in increased levels of congression failure, although only the 7-day treatment was significantly elevated over control values ($\chi^2 = 6.21$; $p < 0.05$; Figure 5)

Discussion

Based on previous studies of human oocytes and of mouse mutants, we hypothesized that congression failure in the mammalian oocyte is causally related to aneuploidy and that it results from endocrine disturbances that affect the final stages of oocyte growth [8, 9]. Thus, the unexpected finding that exposure to BPA, a man-made substance with estrogenic properties, induces both a dramatic increase in congression failure and meiotic aneuploidy in mouse oocytes provides serendipitous validation of this hypothesis.

Many previous studies have suggested that BPA and other man-made substances that mimic estrogens have

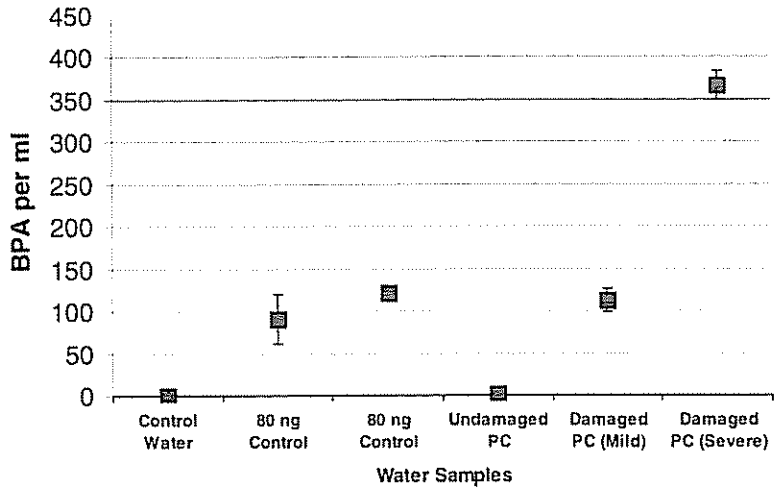

Figure 4. Measurement of BPA in Water Samples

BPA concentrations in water from damaged bottles were determined by gas chromatography-mass spectrometry using electron impact ionization [37]. Test samples included a distilled water control (control water), two samples spiked with 80 ng/ml BPA (80 ng control), one sample from an undamaged polycarbonate bottle (Undamaged PC), one sample from a damaged polycarbonate bottle (Damaged PC [mild]), and one sample from a damaged and leaking polycarbonate bottle (Damaged PC [severe]). To approximate normal operating conditions, undamaged and damaged bottles were filled with 500 ml water, autoclaved by using standard facility procedures (i.e., 60 min on a liquid setting), and allowed to sit at room temperature for 24 hr before samples were acquired. The average concentration and standard deviation for two replicates of each sample type have been plotted on a ng/ml scale, and the level in the control water sample was set to zero. Note that the standard deviation is not apparent for one of the two 80 ng samples, as the replicate values were virtually identical. A slight variation in the 80 ng/ml control values likely reflects the fact that BPA is only moderately soluble in water at neutral pH [37].

adverse effects on mammalian reproduction. However, unlike previous studies that have utilized a prospective toxicological approach (i.e., determining the level of exposure necessary to produce a measurable effect), our studies were a retrospective attempt to understand a sudden change in experimental data. Indeed, the type of environmental accident that precipitated the present studies is, to our knowledge, unprecedented in animal husbandry. In addition to the difference in impetus, the present study differs in two other important respects. First, the abrupt changes in experimental data in two separate studies in our laboratory provide evidence of a striking new reproductive effect; namely, immediate and extreme alterations in the meiotic process in intact animals inadvertently exposed to BPA. Second, unlike many previously reported effects of BPA on mammalian reproduction (e.g., morphological alterations of the reproductive tract [13, 19–22], accelerated onset of puberty [23], disruption of estrus cycles [24], or reduced sperm counts [19, 25]), a defect in meiosis directly influences the genetic quality of the gametes and thus impacts the next generation. Indeed, other data from our laboratory provide evidence that BPA elicits just such a transgenerational effect. In independent studies conducted in the same mouse facility, two nonmosaic chromosome abnormalities (one autosomal trisomy and one unbalanced structural rearrangement) were observed among 16 fetuses karyotyped during the time of maximal exposure. Such abnormalities are extremely rare in our colony and in the literature and seem unlikely to have arisen coincidentally.

Thus, these lines of evidence suggest that, in the mouse, BPA adversely affects chromosome segregation. Clearly, we do not know if this liability extends to humans. Nevertheless, the meiotic program is extraordinarily conserved, and the results of our studies in mice are disturbing because brief exposures during the final stages of oocyte growth were sufficient to cause significant increases in meiotic abnormalities. Further, relatively modest BPA concentrations (i.e., 0.02–0.04 mg/kg body weight/day) elicited significant meiotic effects. Moreover, although the vast majority of our studies were conducted on the first wave of growing follicles in the sexually immature female, a dramatic increase in nondisjunction levels was observed in oocytes from 6- to 8-month-old females during the initial exposure. Thus, sensitivity does not appear to be limited to the prepuberal female.

A number of important questions remain. For example, although our studies suggest that exposure during the final stages of oocyte growth induces meiotic abnormalities, they also raise questions about the critical exposure period. For example, daily oral doses of BPA yielded comparatively low levels of aneuploidy (Table 3; Figure 5), despite our attempts to mimic the exposure dose from damaged caging materials by measuring BPA levels in water from damaged bottles. Possibly, our estimate of initial exposure levels was in error. However, since the level of congression failure in the oral dosing experiments increased both with dose and with exposure time (e.g., Table 3; Figure 5), it seems more likely that the difference is a pharmacokinetic one; i.e., single low oral doses have been reported to be subject to rapid first-pass elimination [17, 18]. Thus, the effect of a more evenly distributed exposure from drinking BPA-contaminated water throughout the day and in association with food intake may be markedly different. It is also possible that BPA acts on meiosis at different developmental stages. For example, an effect that influences the prenatal events of meiotic prophase (i.e., pairing, synapsis, and recombination) might also be evident as an increase in aneuploidy. Alternatively, neonatal exposure might

Table 3. Meiotic Effects of Oral BPA Administration

BPA Dose	Congression Failure
Control: 0 ng/g	2/115 (1.7%)
20 ng/g	10/172 (5.8%)
40 ng/g	19/255 (7.5%)
100 ng/g	5/46 (10.9%)

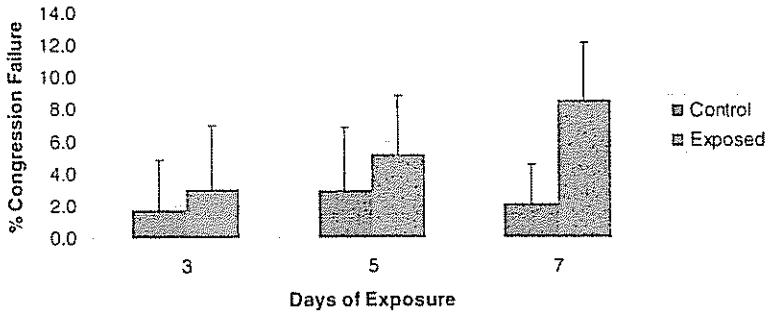

Figure 5. Congression Failure following Oral BPA Administration

Congression failure levels (and 95% confidence intervals) among MII-arrested oocytes from control and BPA-exposed females treated with 20 ng/g body weight BPA in an oil carrier for 3–7 days prior to oocyte collection. The numbers of oocytes examined for the 3-, 5-, 7-day exposures, respectively, were 67, 138, and 234 for treated females and 61, 70, and 140 for control females. While congression failure levels were increased among treated animals for all time points, only the 7-day exposure comparison reached statistical significance ($p < 0.05$).

act to alter the hypothalamic-pituitary-gonadal axis and thus may provide another route to aneuploidy.

The mechanism by which BPA exerts its effect on meiosis remains obscure. Studies of somatic cells exposed in vitro to BPA have demonstrated disturbances in microtubule organization, leading to aneuploidy [26–29]. The effect on the oocyte, however, is likely much more complicated. In the growing follicle, multiple layers of granulosa cells completely envelop the oocyte, and only the innermost layer is in direct contact with the oocyte via gap junctions. Thus, the effect on the oocyte is almost certainly an indirect one, acting through estrogen-mediated changes in the somatic cells of the follicle.

The role of estrogens in folliculogenesis is currently the topic of considerable research interest [30]. The theca cells of the late-stage follicle not only convert testosterone to estrogen, but also express both the ERα and ERβ isoforms of the estrogen receptor [31], whereas the granulosa cells express predominantly ERβ receptors [31]. Thus, estrogenic compounds (e.g., BPA) present in the microcirculation of the ovary have the potential to affect both cell types but likely predominantly those expressing ERβ, since BPA has a higher binding affinity for ERβ than ERα [32]. The importance of estrogen during the late stages of follicle growth has been demonstrated by the reproductive phenotype of targeted disruptions of both estrogen receptors and of the aromatase gene necessary for the conversion of androgens to estrogens (reviewed in [30]). Further, the recent report demonstrating that administration of BPA reverses the reproductive phenotype of the aromatase knockout female provides evidence that the somatic cells of the follicle can be influenced by oral doses of BPA [33].

Currently, there is considerable uncertainty about safe levels of BPA exposure. Plastic industry sources suggest no safety issues related to low-level BPA exposure (e.g., http://www.bisphenol-a.org). Nevertheless, governmental agencies continue to revise safe exposure levels on the basis of animal studies. For example, The European Commission's Scientific Committee on Food recently revised its Tolerable Daily Intake (TDI) from the 0.05 mg/kg body weight level set in 1986 to 0.01 mg/kg body weight (http://europa.eu.int/comm/food/fs/sc/scf/index_en.html). Uncertainty about "safe" exposure levels reflects conflicting reports about the effect of low-dose exposures and differences based on genetic background or route and timing of exposure (e.g., [34–36]). In part, this is due to the lack of a sensitive, reliable, and reproducible assay system. The dose-dependent response observed in our studies suggests that the oocyte and its meiotic spindle may provide just such an assay system for the study of reproductive toxins.

Of more immediate concern, however, we have observed meiotic defects in mice at exposure levels close to or even below those considered "safe." Furthermore, a recent study of pregnant women and their fetuses conducted in Germany suggests that current human exposure levels may well be within this range [21]. Clearly, the possibility that BPA exposure increases the likelihood of genetically abnormal offspring is too serious to be dismissed without extensive further study.

Conclusions

The inadvertent exposure of mice in our colony to bisphenol A provided evidence that this estrogen mimic disrupts chromosome behavior in the mammalian oocyte and causes a specific meiotic phenotype at metaphase (congression failure) and an increased risk of nondisjunction at anaphase. Experimental studies conducted to understand this effect indicate that short-term, low-dose exposure to bisphenol A is sufficient to elicit these meiotic abnormalities.

Our studies have obvious relevance to the genesis of meiotic aneuploidy in the human. Specifically, they are consistent with the hypothesis that endocrine changes affecting oocyte growth underlie human age-related increases in nondisjunction. In addition, these findings raise concerns about the potential reproductive impact of environmental substances that mimic the actions of endogenous hormones.

Experimental Procedures

Oocyte Collection and Analysis

Oocytes were collected and cultured by using conventional laboratory techniques as described previously [8]. Unless otherwise specified, germinal vesicle (GV)-stage oocytes were harvested from 28-day-old females; the first wave of oocytes to initiate growth has reached the antral stage by this time, and a large cohort of meiotically competent oocytes can be obtained. For both congression failure and aneuploidy analyses, the scoring of all preparations was conducted by two independent observers who were blinded with respect to the status (control versus treated) of the specimens.

Congression Failure

Studies conducted at the time of the initial change in control data involved the analysis of the first meiotic division. For these studies,

GV-stage oocytes were liberated from antral follicles, cultured for 8–10 hr, embedded in a fibrin clot attached to a microscope slide, and fixed in a 2% formaldehyde fixative as described previously [8]. Subsequent studies utilized MII-arrested oocytes, since this static arrest phase alleviates the problem of variation due to differences in cell cycle rate. For these studies, GV-stage oocytes were cultured overnight, and only those exhibiting a polar body the following morning were embedded in fibrin clots and fixed. For the analysis of all preparations, slides were immunostained with tubulin antibodies to visualize the spindle and counterstained with DAPI or propidium iodide to visualize the chromosomes as described previously [8].

Aneuploidy Assessment

Studies conducted at the time of the initial change in control data involved the analysis of MII-arrested oocytes from control and inversion carrying females. Air-dried chromosome preparations were made by using a modification of the Tarkowski technique as described previously [8].

Cage and Water Bottle Testing

To assess the effect of damaged polycarbonate caging materials, oocytes were analyzed from normal females born and raised in test and control cages. Siblings were used to establish trio matings (i.e., two females and one male). Test cages consisted of new high-temperature polycarbonate (polyphthalate carbonate) cages exposed to a single contact with either a dilute (1:64) solution ("mild" damage) or full strength ("severe" damage) A-33 detergent (Airkem Professional Products, Ecolab), the detergent responsible for the damage to caging materials in the original animal facility. Following exposure, both mild and severely damaged cages were subjected to 3–4 rounds of thorough rinsing in water, followed by autoclaving before animals were introduced. Subsequently, all caging materials were washed by hand without detergent, and the number of times individual cages were autoclaved for sterilization purposes was recorded by using a cage-marking system. To avoid possible cross-contamination of undamaged caging materials in the course of normal handling, damaged materials were autoclaved separately, and polysulfone cages were used for control matings. In the case of conventional polycarbonate bottles (i.e., nonautoclavable polycarbonate), bottles were simply washed in water following exposure to a dilute (1:64) solution of A-33 detergent. In all cases, test and control matings were run in duplicate.

Measurement of BPA in Water Samples

To determine BPA concentrations in water samples, gas chromatography-mass spectrometry using electron impact ionization was employed [37]. For the assay, the mass spectrometer was run in ion-monitoring mode, and a stock solution of BPA prepared in absolute ethanol at a concentration of 1.01 mg/ml was used to spike 15 ml aliquots of distilled, deionized water to generate a calibration curve. A minimum of two and a maximum of four extractions were run on each test sample by using injection volumes of 1 μl.

Oral BPA Administration

Oocytes were harvested from juvenile (28-day-old) females treated with oral doses of BPA in a corn oil carrier as described previously [19]. In initial studies (Table 3), juvenile females (20- to 22-day-old) were treated with daily doses of 20, 40, or 100 ng/g body weight for 6–8 days preceding oocyte collection and analysis. In subsequent studies (Figure 5), daily doses of 20 ng/g body weight were administered for 3, 5, or 7 days prior to oocyte analysis. Germinal vesicle-stage oocytes were cultured overnight, and oocytes exhibiting a polar body the following morning were fixed, immunostained, and analyzed by using standard laboratory techniques [8]. No difference in the number of oocytes or the rate of polar body extrusion was detected between control and treated females. All scoring was conducted without knowledge of the status (control versus treated) of the female.

Statistical Analysis

Comparisons of levels of aneuploidy or congression failure between controls and exposed/treated animals were conducted by using straightforward goodness of fit tests. In instances in which multiple comparisons were made, Bonferroni corrections were applied to account for the number of statistical tests.

Acknowledgments

The authors express gratitude to A. Vodicka, E. Millie, L. Woods, and J. Cherry for technical assistance and to E. Freeman, F. vom Saal, and R.S. Hawley for helpful discussions. These studies were supported by National Institutes of Health grants ES1172 (P.A.H.) and HD21341 (T.J.H.), ACS postdoctoral fellowship 96994 (K.E.K.), and a Culpepper Foundation Pilot Initiative grant (P.A.H.).

Received: December 11, 2002
Revised: February 3, 2003
Accepted: February 3, 2003
Published: April 1, 2003

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Note Added in Proof

Following the submission of this manuscript, a study describing the release of BPA from new and used polycarbonate caging was published (Howdeshell, K.L., et al. (2003). Bisphenol A is released from used polycarbonate animal cages into water at room temperature. Environ. Health Perspect., in press. Published online February 5, 2003. 10.1289/ehp.5993). This study demonstrates that new polycarbonate cages leach low levels of BPA and that the level of leaching increases markedly under conditions of normal wear.

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Exhibit E

Reproductive Toxicology
24(2): 2007, in press

Chapel Hill Bisphenol A Expert Panel Consensus Statement: Integration of Mechanisms, Effects in Animals and Potential to Impact Human Health at Current Levels of Exposure

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Running Title: Chapel Hill Consensus Statement on BPA

Key Words: Bisphenol A, *in vitro, in vivo,* rat, mouse, aquatic animal, cancer, low dose, non-monotonic dose-response curves, developmental programming

Abbreviations:

ADHD - attention deficit hyperactivity disorder

BADGE - bisphenol A diglycidyl ether

BIS-DMA - bisphenol A dimethacrylate

BIS-GMA - bisphenol A glycerolate dimethacrylate

BPA – bisphenol A

ER – estrogen receptor

Acknowledgements

Meeting support was provided by NIEHS and NIDCR, NIH/DHHS, the US-EPA and Commonweal. We thank Paul French for assistance with the meeting web site and Albert Kingman for advice during preparation of the manuscript. This manuscript does not reflect US-EPA or NIH agency policy. FvS is supported by NIH grant ES11283.

INTRODUCTION

This document is a summary statement of the outcome from the meeting: *"Bisphenol A: An Examination of the Relevance of Ecological, In vitro and Laboratory Animal Studies for Assessing Risks to Human Health"* sponsored by the NIEHS and NIDCR, NIH/DHHS on the estrogenic environmental chemical bisphenol A (BPA, 2, 2-bis (4-hydroxyphenyl) propane; CAS# 80-05-7). The meeting was held in Chapel Hill, NC, November 28-30, 2006 due to concerns about the potential for a relationship between BPA and negative trends in human health that have occurred in recent decades. Examples include increases in abnormal penile/urethra development in males, early sexual maturation in females, an increase in neurobehavioral problems such as attention deficit hyperactivity disorder (ADHD) and autism, an increase in childhood and adult obesity and type 2 diabetes, a regional decrease in sperm count, and an increase in hormonally mediated cancers, such as prostate and breast cancers. Concern has been elevated by published studies reporting a relationship between treatment with "low doses" of BPA and many of theses negative health outcomes in experimental studies in laboratory animals as well as *in vitro* studies identifying plausible molecular mechanisms that could mediate such effects. Importantly, much evidence suggests that these adverse effects are occurring in animals within the range of exposure to BPA of the typical human living in a developed country, where virtually everyone is exposed to measurable blood, tissue and urine levels of BPA that exceed the levels produced by doses used in the "low dose" animal experiments.

Issues relating to BPA were extensively discussed by five panels of experts prior to and during the meeting, and are summarized in five reports included in this issue: 1) Human exposure to bisphenol A (BPA) [1], 2) *In vitro* molecular mechanisms of bisphenol A action [2], 3) *In vivo* effects of bisphenol A in laboratory animals [3], 4) An ecological assessment of bisphenol-A: Evidence from comparative biology [4], 5) An evaluation of evidence for the carcinogenic activity of bisphenol A [5].

Further discussion occurred at the meeting where participants from the panels were reorganized into four breakout groups. The consensus statements from the meeting are presented below.

The definition of "low dose" of BPA at this meeting used the same two criteria established at a prior NIH meeting concerning the low dose endocrine disruptor issue [6]: 1) for laboratory animal studies "low doses" involved administration of doses below those used in traditional toxicological studies conducted for risk assessment purposes. For BPA the lowest dose previously examined for risk assessment purposes was 50 mg/kg/day in studies with rats and mice. The 50-mg/kg/day dose is the currently accepted lowest adverse effect level (LOAEL) that was used to calculate the current US-EPA reference dose (the daily dose that EPA calculates is safe for humans over the lifetime) of 50 μg/kg/day. The current reference dose is thus based on "high dose" experiments conducted in the 1980s [7]. 2) "low dose" also refers to doses within the range of typical human exposure (excluding occupational exposures). For purposes of this meeting, the published literature that was reviewed met both of these criteria for being considered within the "low-dose" range.

Hundreds of *in vitro* and *in vivo* studies regarding the mechanisms and effects of low doses of BPA, as well as studies of bio-monitoring and sources of exposure, have been published in peer-reviewed journals over the last 10 years, since the first "low dose" BPA *in vivo* studies were published [8-10]. The meeting was set up specifically to integrate this relatively new information. This task required the combined expertise of scientists from many different disciplines, and care was taken to ensure that participants covered these diverse areas.

BPA is a high-volume (> 6-billion pounds per year) production chemical used to make resins and polycarbonate plastic [11]. Of particular concern is the use of BPA in food and beverage plastic

storage and heating containers and to line metal cans. In addition, potential environmental sources of BPA contamination are due to use in dental fillings and sealants [12], losses at the production site [13], leaching from landfill [14, 15], and presence in indoor air [16].

BPA has become a chemical of "high concern" only in recent years, even though BPA was shown to stimulate the reproductive system in female rats and thus to be an "environmental estrogen" in 1936 [17], long before it was used as the monomer to synthesize polycarbonate plastic and resins in the early 1950s. However, more recent evidence has shown that BPA also exhibits other modes of endocrine disruption in addition to binding to estrogen receptors, such as alterations in endogenous hormone synthesis, hormone metabolism and hormone concentrations in blood. BPA also results in changes in tissue enzymes and hormone receptors, and interacts with other hormone-response systems, such as the androgen and thyroid hormone receptor signaling systems. While BPA was initially considered to be a "weak" estrogen based on a lower affinity for estrogen receptor alpha relative to estradiol [18], research shows that BPA is equipotent with estradiol in its ability activate responses via recently discovered estrogen receptors associated with the cell membrane [19-22]. Via these receptors, BPA stimulates rapid physiological responses at low picogram per ml (parts per trillion) concentrations.

PURPOSE AND ORGANIZATION OF THE BPA MEETING

Topic-Focused Expert Panels

To address the strength of the evidence regarding the published BPA research, an organizing committee was formed, and five panels of experts from different disciplines were established. Each panel had a chair or co-chairs and included a scientist who agreed to be primarily responsible, along with the chair, for preparing a preliminary draft of the panel's report. A web site was established on

which all of the available electronic files of articles concerning BPA were posted, along with other pertinent information relating to the meeting. Prior to the meeting, the panel members began working on draft reports and communicated via electronic media and telephone conference calls. The resulting preliminary report from each panel was posted on the web site and distributed at the meeting for all participants to read. After the meeting, each panel completed a manuscript that is a part of this meeting report. These five panel reports were peer reviewed using the normal manuscript submission process to *Reproductive Toxicology*.

The following specific concerns about BPA led to the five expert panels being established:

1) Leaching of BPA occurs from the resin lining of metal cans and from plastic food and beverage containers under conditions of normal use. BPA is also detected in water and air samples.
2) Parts per billion (ppb) levels of BPA that are unconjugated (not metabolized and thus biologically active) are detected in human blood and tissues in different countries, and these levels appear to be higher than blood levels that would be present in animals exposed to the US-EPA reference dose.
3) BPA causes a wide range of adverse effects at "low doses" that are below the US-EPA reference dose in animals, both terrestrial and aquatic.
4) There is evidence from *in vitro* mechanistic studies that indicates the potential for disruption of human and animal cell function at concentrations of BPA far below unconjugated levels typically found in human blood and tissues.
5) There is evidence that at very low doses, BPA may be carcinogenic or increase susceptibility to cancer in animals.

The five panels each addressed a different topic related to their specific area of expertise with BPA and prepared a panel report that included documentation of the relevant published studies:

Panel 1) Sources and amounts of human exposure to BPA as well as pharmacokinetics;

Panel 2) *In vitro* studies related to the molecular mechanisms that mediate responses to BPA with an emphasis on studies using low doses;

Panel 3) *In vivo* studies of BPA at "low doses" in laboratory animals;

Panel 4) I*n vivo* studies of BPA in aquatic wildlife and laboratory animals;

Panel 5) Relationship of BPA to cancers.

The purpose of the 3-day meeting was to provide an opportunity for members of the different panels to interact with each other to integrate information from different disciplines concerning low-dose effects of BPA after each panel of experts had prepared a report in its specific area. The agenda of the meeting was designed to allow the members of the five panels to have time to discuss the information in their panel reports and finalize statements about the strength of the evidence for the literature that the panel had reviewed.

Integration of Information by Breakout Groups

For the second part of the meeting the focus was on integrating the information from each of the panel reports. This was accomplished by assigning panel members to one of four breakout groups. The four replicate breakout groups were established using the following criteria, such that each breakout group should have:

1) At least two members from each of the five panels;

2) A person from each panel who had published on BPA;

3) A person with general knowledge of endocrine disruption research or endocrinology, but who

had not necessarily published on BPA;

4) A person with experience in the process of reaching consensus;

5) A mixture of junior and senior investigators.

The charge to the replicate breakout groups was to individually integrate the information relating to the following four issues:

Issue 1) Determine the degree to which the findings on BPA mechanisms of action identify mechanisms and bioactive doses that explain results of the studies reported by the panel on *in vivo* laboratory animal studies. Determine the strength of the evidence for plausible mechanisms mediating *in vivo* effects at low doses. In addition, identify any *in vivo* findings that are unexpected based on the *in vitro* literature.

Issue 2) Assess the degree to which ecological studies with wildlife are consistent with laboratory studies in similar and different species. For example, determine the similarity of exposure levels and types of responses seen in wildlife and laboratory animals.

Issue 3) Discuss the degree to which the low doses of BPA used in laboratory animal studies relate to the levels detected in human serum and tissues (including urine).

Issue 4) Assess the importance of life stage in the pharmacokinetics of BPA, levels of exposure to BPA, and the health effects of BPA in animals and humans.

FINDINGS SUBMITTED BY THE FOUR BREAKOUT GROUPS

The reports from the breakout groups are presented below. The four breakout groups conducted a critical examination of the published research on BPA in relation to the four topics described above. Each of the breakout groups identified areas of knowledge and research gaps and made suggestions

for future directions of research. In addition, each group identified which of the following two categories applied to specific outcomes:

- "We are confident of the following": This category applied when there were findings reported in multiple papers from multiple labs that were in agreement. There should have been no papers reporting conflicting findings, unless there were flaws in those papers, in which case the flaw(s) should have been identified.
- "We believe the following to be likely but requiring confirmation": This category applied when there were multiple consistent findings from one lab, or there may have been some conflicting reports along with reports of significant findings.

LEVELS OF CONFIDENCE FOR PUBLISHED BPA FINDINGS

The responses from the four different breakout groups were integrated together and organized based on levels of confidence. The criterion for a statement being included in a category was that there had to be consensus among all four of the breakout groups about the statement.

A. Based on existing data we are confident of the following:

Issue 1: *In vitro* mechanistic research – laboratory animal research connection

1. *In vitro* studies have provided two routes of plausibility for low dose *in vivo* effects of BPA. These include binding to nuclear estrogen receptors that regulate transcription as well as estrogen receptors associated with the cell membrane that promote calcium mobilization and intracellular signaling. Receptors associated with the cell membrane are more sensitive to BPA than are nuclear receptors. Actions mediated by membrane associated receptor signaling may underlie much of the low-dose BPA phenomena (effects have been reported at doses as low as 1 pM or 0.23 ppt). This increases

plausibility of effects at low doses, which are within the range of environmentally relevant doses (human and wildlife levels of exposure).

2. *In vitro* mechanistic information has informed us that exposing tissues to only an extremely narrow range of doses of BPA may lead to erroneous conclusions. Non-monotonic dose-response curves are encountered frequently in basic endocrinological research, and numerous examples have been reported for BPA (reviewed in: [18, 23, 24]. Because of this animal experiments on unstudied systems must avoid narrow dose ranges, especially the use of only a few very high doses. Thus, testing one or two doses and concluding that there are no effects is inappropriate. At somewhat higher doses than are required for estrogen receptor (ER)-mediated responses, BPA also interacts with androgen and thyroid hormone receptors, making predictions of effects at different doses very complex.

3. *In vitro* studies can dissect mechanisms of complicated effects observed *in vivo*. The proposed potential mechanisms acting *in vitro* and *in vivo* are the same, involving estrogen receptor mediated (nuclear and membrane-associated) actions. However, specific effects are dose and cell/tissue specific. In addition, there are *in vivo* processes that are not reflective of currently known mechanisms that have been identified *in vitro*. This is due to previously unknown mechanisms as well as the complexity (due to interactions among cell and tissue types) of *in vivo* systems.

Issue 2: Wildlife – laboratory animal research connection

1. BPA is found in the environment: aquatic, terrestrial and air.

2. Studies of wildlife demonstrate estrogenic responses that are similar to responses seen in laboratory animals. Specifically, reductions in spermatogenesis are seen in wildlife at ecological concentrations of BPA, and these effects are also seen in controlled laboratory studies with BPA. In addition, vitellogenin response is a common biomarker in wildlife and non-mammalian laboratory species for BPA-induced estrogen receptor activation as well as activation by other estrogens.

3. BPA exposure induces similar effects in reproductive systems in wildlife and experimental animal model systems, but concentrations used in experiments involving wildlife species are often higher than environmental exposures. There are conditions in the environment, such as landfill leachates and effluent outflow, that cause episodic exposure of field populations to elevated doses of BPA.

4. Responses in a variety of vertebrate wildlife species are qualitatively consistent with controlled laboratory studies with BPA. Thus, animals in the wild show evidence of harm, and controlled laboratory studies with model aquatic animals (i.e. medaka, zebrafish, fathead minnows) are consistent with observations made in wildlife species. Low dose effects of BPA (low ppb range) have been observed in many of these animals.

5. The similar effects observed in wildlife and laboratory animals exposed to BPA predict that similar effects are also occurring in humans.

Issue 3: Laboratory animal research – human exposure connection

1. Human exposure to BPA is widespread.

2. Human exposure to BPA is variable, and exposure levels cover a large range [central tendency for unconjugated BPA: 0.3 – 4.4 ng/ml (ppb)] in tissues and fluids in fetuses, children and adults.

3. Because the current published literature states that there is a linear relationship between administered dose and circulating levels of BPA in animal studies, this allows circulating levels at lower administered doses to be predicted in experimental animals based on the results from studies in which higher doses were administered.

4. All of the currently published metabolic studies in rats predict circulating BPA levels after acute low-dose oral exposures at blood levels less than or equal to 2 ng/ml (ppb), which is the approximate median and mean unconjugated circulating BPA level in humans. Therefore, the commonly reported circulating levels in humans exceed the circulating levels extrapolated from acute exposure studies in laboratory animals.

5. BPA levels in the fetal mouse exposed to BPA by maternal delivery of 25 μg/kg, a dose that has produced adverse effects in multiple experiments, are well within the range of unconjugated BPA levels observed in human fetal blood.

Issue 4: Life stage – relationship to exposure pharmacokinetics, and health effects

1. Sensitivity to endocrine disruptors, including BPA, varies extensively with life stage, indicating that there are specific windows of increased sensitivity at multiple life stages. Therefore, it is essential to assess the impact of life stage on the response to BPA in studies involving wildlife, laboratory animals, and humans.

2. Developmental windows of susceptibility are comparable in vertebrate wildlife species and laboratory animals.

3. BPA alters "epigenetic programming" of genes in experimental animals and wildlife that results in persistent effects that are expressed later in life [25]. These organizational effects (functional and structural) in response to exposure to low doses of BPA during organogenesis persist into adulthood, long after the period of exposure has ended. Specifically, prenatal and/or neonatal exposure to low doses of BPA results in organizational changes in the prostate, breast, testis, mammary glands, body size, brain structure and chemistry, and behavior of laboratory animals.

4. There are effects due to exposure in adulthood that occur at low doses of BPA. Substantial neurobehavioral effects and reproductive effects in both males and females have been observed during adult exposures in laboratory animals.

5. Adult exposure studies cannot be presumed to predict the results of exposure during development.

6. Life stage impacts the pharmacokinetics of BPA.

B. We believe the following to be likely but require confirmation:

Issue 1: *In vitro* mechanistic research – laboratory animal research connection

1. BPA metabolism occurs in cell culture systems, and although there are differences between cell types, there is less variability than in the entire animal. Metabolism is an important issue for humans and wildlife field populations with large genetic variability. Individual differences in BPA

pharmacokinetics allow for underlying variability within a population, and may allow for the identification of sensitive and insensitive subpopulations.

2. The activity of various enzymes involved in drug, chemical and hormone metabolism, as well as protection against oxidative stress, are programmed by hormone levels during sensitive periods in development. Developmental alterations in hormonal programming (activation or inhibition) may thus affect metabolism of BPA and other hormones and chemicals. Direct interaction of BPA with enzymes in cells has only been reported at higher doses than expected for human exposures.

3. The set of genes regulated by different doses of BPA is expected to differ among doses. Therefore, different doses of BPA do not produce different effects only due to a quantitative difference in the expression of the same set of genes.

4. Differential expression of estrogen receptor subtypes (α/ß; variant isoforms), and protein-protein interactions (estrogen receptor homo and hetero dimer formation, co-regulators, etc) modulate the cellular response to BPA. Direct actions of BPA on intracellular signal transduction modulate some cellular responses, which are similarly dependent on differential expression and protein-protein interactions.

5. Bioactive doses can be mathematically modeled, but further model refinement and experimental conformation is required.

6. Other mechanisms (androgen receptors, thyroid hormone receptors) may be relevant for BPA action, but at higher doses than for estrogen responsive mechanisms.

Issue 2: Wildlife – laboratory animal research connection

1. The effects observed in laboratory animals could be present in wildlife, because the low doses being studied in laboratory animals are now relevant to environmental exposure levels of wildlife. The similarities in mechanisms that have been observed between different species suggest that field populations will respond to the same low levels.

2. Measurements of vitellogenin production in fish have established that there are exogenous estrogenic signals in the their environment. BPA may be contributing to this phenomenon as it enters natural water systems after leaching from landfills and due to plastic debris in water.

3. Delayed spawning is seen in male and female fish, which may relate to observed changes in estrous cyclicity in mammals in laboratory experiments.

4. In wildlife and laboratory studies, BPA induces alteration in steroid biosynthesis/ metabolism/excretion.

5. Wildlife residing in sediment is likely exposed to higher levels of BPA.

Issue 3: Laboratory animal research – human exposure connection

1. Human exposure is likely to be continuous, unlike exposure in most all laboratory animal studies of BPA pharmacokinetics.

Issue 4: Life stage – relationship to exposure pharmacokinetics, and health effects

1. Clearance of BPA in the fetus is reduced compared to other life stages. Different effects and metabolic clearance mechanisms are also observed in neonatal and adult animals. Conjugation (glucuronidation) and other mechanisms of metabolic clearance of BPA thus vary throughout life.

2. Exposure to BPA during different life stages differentially influences reproductive cancer etiology and progression, and exposure during sensitive periods in organogenesis may increase susceptibility to development of cancers in some organs, such as the prostate and mammary glands.

4. Early life exposure to environmentally relevant BPA doses may result in persistent adverse effects in humans.

5. The function of the immune system can be altered following adult exposure to BPA.

6. Effects on insulin metabolism occur following adult exposure.

C. Areas of uncertainty and suggestions for future research

Issue 1: *In vitro* mechanistic research – laboratory animal research connection

1. Since BPA can act as an agonist or an antagonist in different tissues and against different background physiological states, the specific co-regulators that mediated these different responses of BPA need to be elucidated based on *in vitro* mechanistic studies, which should be confirmed *in vivo*.

2. Research is needed on specific receptor sub-types (i.e., classical nuclear and non-classical membrane-associated estrogen receptors) in relation to the potency of BPA in different tissues.

3. The identification of multiple estrogen receptor genes and variants as well as different co-regulators with different activities reveals that different levels of potency of BPA could be obtained by complex interactions between these different components that would not be predicted in homogeneous recombinant systems.

Issue 2: Wildlife – laboratory animal research connection

1. To directly relate the effects seen in wildlife with BPA exposure, biomonitoring data are needed from wildlife. In addition to BPA levels, these studies should assay total estrogenic and antiandrogenic activity from other contaminants.

2. There is a need to examine sensitive endpoints in wildlife that have been identified in laboratory animals.

3. There are substantial amounts of plastic debris within marine and fresh water ecosystems, and studies are needed to examine the impact of BPA in the environment on aquatic organisms. Doses used in laboratory experiments involving wildlife should reflect environmental exposures.

4. More studies need to be done with BPA in invertebrates, and a fundamental understanding of estrogen action in invertebrates is required.

5. Studies should determine if amplification of BPA through the food chain occurs, particularly under anaerobic or hypoxic conditions due to the lack of microbial or photodegradation.

6. Future research emphasis should be placed on populations of aquatic animals exposed to landfill leachate and sewage effluent, as these are the primary point sources for BPA exposure.

Issue 3: Laboratory animal research – human exposure connection

1. Even though there have been attempts to estimate daily human intake of BPA, these estimates require many assumptions. The best measures we have to estimate whether humans may be affected by current exposures to BPA are levels in blood (not exposure levels), which can be related to blood levels in experimental animals after acute exposures. Known sources of human exposure to BPA do not appear sufficient to explain levels measured in human tissues and fluids.

2. While BPA is not persistent in the environment or in humans, biomonitoring surveys indicate that exposure is continuous. This is problematic because acute animal exposure studies are used to estimate daily human exposure to BPA, and at this time, we are not aware of any studies that have examined BPA pharmacokinetics in animal models following continuous low-level exposures. Measurement of BPA levels in serum and other body fluids suggests that either BPA intake is much higher than accounted for, or that BPA can bioaccumulate in some conditions such as pregnancy, or both. Research using both animal models, as well as epidemiology studies, are needed to address these hypotheses, and this research needs to better mimic the apparent continuous exposure of humans to BPA.

3. More comprehensive exposure and biomonitoring studies are needed, especially in developing countries.

4. In both animal and human studies, internal exposure measures need to be related to health effects. In particular, there is a need for epidemiological studies relating health outcomes to BPA exposure, particularly during sensitive periods in development. These studies should be based on hypotheses from findings in experimental animals. This will require additional development of appropriate biomarkers in animal studies that can be used in epidemiological research.

Issue 4: Life stage – relationship to exposure pharmacokinetics, and health effects

1. While there is a great need to continue studying prenatal and perinatal exposures in laboratory animal studies, many organs and endpoints continue developing at later stages (throughout puberty and adolescence). Additional studies are needed during these later periods of development.

2. Additional research is needed regarding exposure to BPA in adulthood to determine whether when exposure has ended, effects are or are not permanent and associated with subsequent age-related diseases.

3. Because aging adults lose repair mechanisms, metabolic enzymes, and imprinted genes, the possibility that adult exposures (long-term, low level) can increase the risk of cancers and other conditions during aging should be addressed with additional human research and the development of appropriate animal models.

4. Epigenetics should be examined as a potential mechanism mediating developmental effects as well as the trans-generational effects of BPA and other contaminants. Potential effects of adult exposures also need to be examined in relation to disruption of epigenetic changes that occur normally during aging.

5. Trans-generational and multi-generational effects of BPA must be examined in laboratory animals and humans.

6. There is a need for studies that involve collection of human blood and urine from humans at several life stages, with specific emphasis on infants and young children and continued monitoring throughout adulthood. Additionally, there is a need to characterize the basis for the variability in BPA levels in studies examining both human urine and serum.

7. There is a need for research on the genetic basis for differences in susceptibility to BPA and other contaminants.

8. Studies are needed on comparative BPA pharmacokinetics in invertebrates and vertebrates (non-human primates included).

9. There is a need to measure total endocrine disrupter load in humans and wildlife. Therefore, biomarkers of endocrine disrupter exposure are necessary.

10. There is a need for more research directed at examining human exposure, pharmacokinetics and health effects of selected BPA precursors (i.e., BADGE, BISGMA, BISDMA) and metabolites (e.g., halogenated BPAs).

11. There is a need for more studies focused on identification of other (non-estrogen-receptor mediated) mechanisms of action of BPA.

12. Effects of chemicals on the immune system is life stage dependent, and identifying the life stage dependency for BPA effects on the immune system is necessary. In addition, studies examining BPA effects on the immune system in wildlife are necessary.

CONCLUSIONS

The published scientific literature on human and animal exposure to low doses of BPA in relation to *in vitro* mechanistic studies reveals that human exposure to BPA is within the range that is predicted to be biologically active in over 95% of people sampled. The wide range of adverse effects of low doses of BPA in laboratory animals exposed both during development and in adulthood is a great cause for concern with regard to the potential for similar adverse effects in humans. Recent trends in human diseases relate to adverse effects observed in experimental animals exposed to low doses of BPA. Specific examples include: the increase in prostate and breast cancer, uro-genital abnormalities in male babies, a decline in semen quality in men, early onset of puberty in girls, metabolic disorders including insulin resistant (type 2) diabetes and obesity, and neurobehavioral problems such as attention deficit hyperactivity disorder (ADHD).

There is extensive evidence that outcomes may not become apparent until long after BPA exposure during development has occurred. The issue of a very long latency for effects *in utero* to be observed is referred to as the developmental origins of adult health and disease (DOHaD) hypothesis. These developmental effects are irreversible and can occur due to low-dose exposure during brief sensitive periods in development, even though no BPA may be detected when the damage or disease is expressed. However, this does not diminish our concern for adult exposure, where many adverse outcomes are observed while exposure is occurring. Concern regarding exposure throughout life is

based on evidence that there is chronic, low level exposure of virtually everyone in developed countries to BPA. These findings indicate that acute studies in animals, particularly traditional toxicological studies that only involve the use of high doses of BPA, do not reflect the situation in humans.

The fact that very few epidemiological studies have been conducted to address the issue of the potential for BPA to impact human health is a concern, and more research is clearly needed. This also applies to wildlife, both aquatic and terrestrial. The formulation of hypotheses for the epidemiological and ecologists studies can be greatly facilitated by the extensive evidence from laboratory animal studies, particularly when common mechanisms that could plausibly mediate the responses are known to be very similar in the laboratory animal models, wildlife and humans.

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Exhibit F

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JOURNAL SENTINEL WATCHDOG REPORT

Are your products safe? You can't tell.

Labels often fail to list compounds that can disrupt biological development

By SUSANNE RUST, MEG KISSINGER and CARY SPIVAK
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Posted: Nov. 24, 2007

Take a look at your shoes, your shampoo, your carpet.

Your baby's bottles, even the dental sealants in your mouth.

These products contain chemicals that disrupt the natural way hormones work inside of you.

The chemicals known as endocrine disruptors are all over your house, your clothing, your car.

The chemicals are even in you.

They promise to make skin softer, clothes smell fresher and food keep longer.

The problem is, neither the companies that make these products nor federal regulators are telling you that some of these substances may be dangerous. Many have been found to cause life-threatening illnesses in laboratory animals.

Chemical makers maintain that their products are safe. They point to government assurances and the millions of dollars they have spent on their own research as proof.

But a growing number of scientists are convinced the chemicals interfere with the body's reproductive, developmental and behavioral systems.

Hundreds of studies have shown that these compounds cause a host of problems in lab animals. They include cancers of the breast, brain and testicles; lowered sperm counts, early puberty, miscarriages and other defects of the reproductive system; diabetes; attention deficit disorder, asthma and autism - all of which have spiked in people in recent decades since many of these chemicals saturated the marketplace.

A Journal Sentinel investigation found that the government has failed to regulate these chemicals, despite repeated

promises to do so. The regulatory effort has been marked by wasted time, wasted money and influence from chemical manufacturers.

The newspaper reviewed more than 250 scientific studies written over the past 20 years; examined thousands of pages of regulatory documents and industry correspondence; and interviewed more than 100 scientists, physicians, and industry and government officials.

Among the findings:

• U.S. regulators promised a decade ago to screen more than 15,000 chemicals for their effects on the endocrine system. They've spent tens of millions of dollars on the testing program. As yet, not a single screen has been done.

• Dozens of chemicals the government wants to screen first have already been tested over and over, even while thousands of untested chemicals are waiting to be screened.

• By the time the government gets around to doing the testing, chances are the results will be outdated and inconclusive. The government's proposed tests lack new, more sensitive measures that would identify dangerous chemicals that older screens could miss.

• As the U.S. testing process remains grounded, hundreds of products have been banned in countries around the world. Children's products - including some baby toys and teething rings - outlawed as dangerous by the European Union, Japan and Canada, are available here without warning.

• Lacking any regulation in the U.S., it's impossible for consumers to know which products are made with the dangerous compounds. Many companies don't list chemicals known to disrupt the endocrine system on product labels.

The government's efforts have been "an abject failure, a disaster," said Philip Landrigan, a pediatrician and chairman of the department of community and preventive medicine at Mount Sinai School of Medicine in New York.

Landrigan was at the White House ceremony in 1996 when President Clinton signed laws requiring the U.S. Environmental Protection Agency to screen chemicals for their effects on the endocrine system.

Because the effects of endocrine disruptors may take years to reveal themselves, it is almost impossible to say that a particular chemical caused a certain disease. There also is a lot of uncertainty about how these chemicals work inside your body. So, scientists extrapolate. They can't test their theories on humans. Instead, they have to rely on animal studies and try to figure out the implications for people.

By mimicking or blocking the body's hormones, endocrine disruptors can trigger faulty messages that disrupt development. That makes them particularly dangerous to fetuses and young children, scientists say. These chemicals can be ingested, inhaled and absorbed through the skin.

Michael E. Mitchell, chief of pediatric urology at Children's Hospital of Wisconsin, has seen the consequences he attributes to these unregulated chemicals.

He has witnessed a dramatic spike in the number of genital birth defects in the last 30 years. And it breaks his heart, he said, to see the damage done to so many children who must undergo painful surgery to correct birth deformities.

Considering the number of chemicals that developing fetuses are exposed to, "it's amazing that anyone turns out OK," he said.

Anxiety is rising over the growing number of cancer cases and other diseases linked to these chemicals. But few answers are forthcoming.

"People should know what they're being exposed to and be given the option to choose alternatives," said Shanna Swan, director of the Center for Reproductive Epidemiology at the University of Rochester School of Medicine and Dentistry. "And that is not happening very fast."

EPA officials blame their lack of progress on the complexity of the undertaking.

"Clearly, we would have liked to have been a lot further along," said Elaine Francis, national program director of the EPA's endocrine disruptors research program. "But science tends to move at its own pace."

To find how pervasive these compounds are in everyday use, the Journal Sentinel asked Frederick vom Saal, an internationally known expert in endocrine disruption, to perform a chemical audit of the Greendale home of Dean and Ellen Lang Roder and their four children, ages 3 to 10.

As the University of Missouri biologist went through each room in the house, vom Saal found hundreds of reasons for the Roder family to worry - from the bathtub rubber duck to the plastic pipes that bring water into their home.

"Anything that goes in your child's mouth is a factor for you to be concerned about," vom Saal told Ellen Roder as he held one of her children's dolls. "Particularly, dolls made from a plastic called polyvinyl chloride that 10 years from now just won't exist. It will be looked at like cigarettes. It is that dangerous."

Industry scientists dispute that.

"Science supports our side," said Marty Durbin, federal affairs managing director for the American Chemistry Council, the trade group representing the plastics industry.

They say there is no reason to fear the toys, baby bottles and other products containing the chemicals because none of their studies has proved that the chemicals cause harm to people. Chemists for the industry say you would have to consume 1,300 pounds of canned and bottled foods each day to notice any effects from the chemicals those products contain.

"I'm very comfortable with my kids and grandkids using these products, and that's really my bottom line," said James Lamb, an industry consultant and former EPA regulator. "And it is because I believe the industry has done the studies that need to be done and that they're interpreting them properly."

Lack of screening

There are roughly 100,000 chemicals on the market today. Yet, lacking a coordinated screening program, there is no way to know how many of these chemicals interfere with the human endocrine system.

The chemicals at issue are used as additives in plastics, fragrances, creams and as flame retardants.

Some of the more controversial compounds include bisphenol A and certain phthalates.

Six billion pounds of bisphenol A, the raw material of polycarbonate plastic and epoxy resins, are produced each year in the United States.

Phthalates (pronounced "THAL-ates") are the chemicals that make plastic flexible and allow creams and personal-care

products to hold their smell. U.S. chemical companies produce more than 2 billion pounds of these compounds a year. They are commonly found in nail polishes and hair sprays, shower curtains and even Halloween costumes.

For more than a decade, government agencies have said that several of these chemicals are safe at levels that people are exposed to every day.

Chemical makers have relied on these assurances as proof that their products are safe. They bolster these conclusions with millions of dollars of research and testing.

But the newspaper's review of 258 studies of bisphenol A, a common ingredient in baby bottles, reusable water bottles, eyeglass lenses and DVDs, shows otherwise.

More than 80% of studies analyzed by the Journal Sentinel show that the chemical adversely affects animals, causing cancer and other diseases.

Developing embryos exposed to endocrine disruptors through their mothers are most at risk, said Theo Colborn, a scientist trained at the University of Wisconsin-Madison whose book on the explosion of dangerous chemicals in the environment, titled "Our Stolen Future," stirred passionate calls for reform and regulation when it was published in 1996.

"You need the right hormones in the right place at the right time sending out the right signals," Colborn said. "If that's fouled up prenatally, you're in trouble."

Colborn, like many of her colleagues, has changed the way she deals with these compounds, refusing to store her food in plastic or use certain creams and lotions that contain chemicals suspected of causing harm.

Wildlife abnormalities

Scientists first suspected that endocrine disruptors were wreaking havoc decades ago when they began observing freakish abnormalities in wild animals, particularly along the Great Lakes with its legacy of industrial pollution.

They were seeing female gulls nesting together, birds with twisted bills and frogs with severe deformities, including one with an eye growing inside its mouth. Elsewhere across the country, scientists reported finding male fish with sacks of eggs and alligators with withered penises.

In 1991, Colborn, then a zoologist working for the World Wildlife Fund, convened a conference of some of the country's leading wildlife biologists, toxicologists and endocrinologists at Wingspread Conference Center in Racine to discuss the emerging science.

It was there that the term "endocrine disruptor" was coined. The 21 scientists signed a consensus statement, expressing concern about the dangers that these new chemicals posed and calling for them to be tested immediately.

Five years later, Colborn and two colleagues chronicled the bizarre spectacles of nature and their theories about the causes.

The authors wondered that if the toxins in the environment could cause these effects in animals, what were they doing to people? Just as with lead and tobacco decades before, these chemicals are all around us, ravaging nature's delicate design, the authors said.

Their book stirred controversy in the scientific community, and many dismissed the claims as "junk science" because

there was no direct link between specific chemicals and illnesses in people.

Within days of the book's publication, the chemical industry's trade group issued an alert to its members, warning them to expect a swarm of calls about the book's claims. The memo predicted the fallout could be fierce.

It was.

Later that year, Congress unanimously passed two laws ordering the EPA to begin screening and testing chemicals and pesticides for endocrine disrupting effects by 1999.

The EPA convened a committee of scientists from academia, the government and the chemical industry to lay the groundwork for testing these chemicals. They came up with a way to identify and test chemicals for the risks and get the information to the public.

In the beginning, there was a groundswell of enthusiasm. Then-EPA administrator Carol Browner said in 1998 that her agency would begin fast-tracking efforts to screen these compounds by the end of that year.

"Some 15,000 chemicals used in thousands of common products, ranging from pesticides to plastics," would be screened, Browner said.

Officials identified the program as a top priority. Browner appointed the first panel of scientists to build a framework for how to screen the chemicals. She left the agency after the presidential election in 2000.

More than $80 million later, the government program has yet to screen its first chemical.

That has left Browner, and others, concerned about the lack of any results.

"It doesn't take nine years," she said with a sigh. "You adjust as you go. You don't have to build a Cadillac when a Model T will do."

Promise unfulfilled

Frustrated at the lack of action, a consortium of environmental, patient advocacy and labor groups filed a federal lawsuit, prompting the EPA to promise that screening would begin by the end of 2003.

But the agency repeatedly has missed its self-imposed deadlines as well as those set by law.

Agency administrators testified twice before Congress, first in August 2000 and again two years later, pledging that the screening would be in place soon. Three separate committees of academic and industry scientists, including the one Browner formed, have been appointed by the EPA to take up the issue.

"A lot of bureaucratic foot-stomping and dust-raising," was the observation of Peter DeFur, a researcher at the Center for Environmental Studies at Virginia Commonwealth University who served on all three of the committees.

"To delay is to win on the part of the industrial community," DeFur said.

Industry, he said, tried mightily to slow the effort. He was particularly critical of one test pushed by chemical makers that involved studying mature male rats to see the chemicals' effects on the development of the reproductive system.

"What does the old white rat have to do with development?" DeFur said. "By the time he gets to be mature, or even

nearly mature, all the organs are developed."

Industry and other groups have flooded the EPA and the committees with research, said L. Earl Gray Jr., an EPA research biologist.

The industry's lobbying efforts are led by the American Chemistry Council. The group has a $75 million budget and includes some of the biggest names in commerce - Dow Chemical Corp., Procter & Gamble Co. and DuPont.

Chemical makers have "in some sense learned that if you play on the uncertainty of danger, you're going to be able to stop regulatory action especially in an anti-regulatory era," said David Rosner, professor of history and public health at Columbia University. That's particularly true "in a time when so many of our regulatory agencies have been neutered politically and socially," he added.

Durbin, of the trade group, denied any stall tactics.

"If it was our interest to delay things around here, we'd just sit on our hands and see whether or not EPA gets any funding," said Durbin, noting that the trade group frequently lobbies for increases in the EPA's budget.

Annual federal funding for the endocrine disruptor screening program peaked at $12.6 million in 2000 and has dropped by about one-third.

Critics have charged that the White House has cut back on efforts to regulate a wide array of industries. DeFur, among others, felt that frustration while serving on the endocrine disruptor committees.

Clifford Gabriel, director of the EPA's Office of Science Coordination and Policy, countered that budgetary constraints have not hurt the progress.

Stephen L. Johnson, Browner's successor as head of the EPA, declined requests to be interviewed.

Whatever the reason, the committees met less frequently as time went by.

By April 2006, 10 years after the congressional order to begin the screening, progress stalled altogether.

Gerald LeBlanc, chairman of the committee charged with developing the screens, got a call from an EPA administrator, assuming that the two would be setting the committee's next meeting. Instead, LeBlanc was told the committee was being terminated.

"They were not going to allow me to take this job to completion," said LeBlanc, toxicology professor at North Carolina State University.

Edward Orlando, a biology professor at Florida Atlantic University and a member of the last committee, said its abrupt dissolution came as a disappointment - not to mention a waste of public money.

"How long will this take? Another five years? Another 10?" Orlando said.

The EPA's Francis said that LeBlanc's committee had a set term, and the agency felt it was more efficient to turn the work over to an advisory panel, where it remains today. But committee members say the effort was doomed for the past several years.

"Frankly, there was not enough political oomph behind it," said Gina Solomon, a member of the first EPA committee

and senior scientist for the National Resources Defense Council.

Those with ties to industry say they, too, wish the process moved faster.

"Everyone is disappointed that you can't make quicker progress, but it does take time," said Thomas Osimitz, an industry consultant who sat on two of the three EPA committees. "It's frustrating, but, on the other hand, I don't know what could be quicker."

Outdated testing

By the time the government gets around to the tests, they likely will be of little value. Under the current model, government tests do not screen for the chemicals' effects at low doses.

Instead, government researchers follow standard toxicology testing practices, feeding animals such as rats huge doses of the chemical.

Then they record the damage to the animal, most often cancer, behavioral or reproductive failures. The researchers then test the rats at lower and lower doses until they no longer find those problems.

But bisphenol A and phthalates don't work that way, many scientists say. They can elicit different effects in animals at extremely low doses.

Two groups of scientists, one from the National Academy of Science and the other from the National Toxicology Program, have called for a radical reform in the way that government screens these chemicals. But, so far, the government hasn't budged from its original formula.

"The EPA is lumbering along trying to clumsily incorporate the science of a couple of decades ago," Solomon said.

The list of chemicals scheduled to be screened is also being questioned.

The EPA will first screen 73 chemicals - all pesticides, none of the chemicals found in household products. The tests aren't set to happen until sometime next year.

EPA officials declined to say exactly when the screening would occur, explaining that the agency must finish its study of the tests before shipping them to another panel for review. But most of the pesticides have already been tested, and many have been established as endocrine disruptors.

Francis, of the EPA, says her agency chose to screen that relatively small batch of chemicals as a way to test the reliability of the process. But even scientists hired by the chemical industry question the value of screening chemicals that have been studied thoroughly.

"Most of those on the list have already been tested, so why are we doing this?" asked Lamb, the toxicologist who works as a consultant to the chemistry council.

The EPA hopes to conclude the first round of tests by 2010, said Enesta Jones, an agency spokeswoman. Only then will the agency have an idea when the next group of chemicals will be screened.

Buyer beware

For as slow as the process of screening chemicals has been in the U.S., concern about the safety of endocrine disruptors has caught on in Europe, Japan, South America, the Middle East, Mexico and even Fiji.

Reports of declining sperm counts, birth defects and fertility problems have sparked widespread concern there. The European Union has banned 1,100 chemicals from cosmetics that are thought to cause cancer or reproductive harm.

"When we go to Europe, I breathe a sigh of relief because of all of the things I'm not exposed to over there," said Rochester's Swan, an epidemiologist and biostatistician.

Earlier this year, the European Union passed a law that requires chemical companies to prove their products are safe before they are put on the market.

The U.S. has no such protocol, known as the precautionary principle, and the chemical industry has argued against it.

"The problem with the precautionary principle is that you have a moving target," said Tim Shestek, a chemistry council lobbyist. "You need to prove that something is safe - safe is never really defined by anybody."

Lacking testing or regulation by the U.S. government, it falls to consumers to watch out for themselves.

Buyers must know the names of specific chemicals - such as dibutyl phthalate and diethyl phthalate - if they want to find out if a bottle of nail polish or a jar of hand lotion contains endocrine disruptors.

Even then, if the chemical is not considered a key ingredient, the company is not required to include it on the label.

There is nothing listed on a bottle of Chanel Precision Energising Radiance Lotion, for example, to let you know that it contains at least six chemicals that have been linked in laboratory studies to cancer in animals. Nor can you know by looking at the label for Avon's Anew Ultimate Skin Transforming Cream that it contains chemicals linked to cancer and endocrine disruption, according to a review by the nonprofit Environmental Working Group.

A spokeswoman for Chanel declined comment, and officials from Avon Products Inc. referred questions to the Cosmetic, Toiletry and Fragrance Association, which dismissed the claims as unfounded.

Consumer groups

Consumer interest groups are trying to answer some of the questions that the government is not. The Campaign for Safe Cosmetics, a coalition of groups concerned with women's health, labor, consumer rights and the environment, offers a Web site run by the Environmental Working Group that enables shoppers to check the safety of cosmetics and personal-care products. The site identifies more than 450 products that are banned as dangerous in other countries but are widely available here.

As consumers learn more about these chemicals, more firms are taking steps to remove them from product lines.

Cosmetics giant Revlon Inc., for example, stopped using phthalates 15 years ago. A company spokeswoman said its products, including those sold in the U.S., comply with the stricter rules of the European governments.

Other companies following similar policies include the L'Oreal Group, Hasbro Inc. and McDonald's Corp. In 1998, the fast-food giant stopped using phthalates in its Happy Meal toys designed for children age 3 and younger.

Retailers, including Target Corp. and Whole Foods Market Inc., have removed items and are looking at ways to eliminate products that contain some endocrine disruptors.

"We are committed to reducing PVC in our products and packaging," said Susan Kahn, a vice president at Target, referring to polyvinyl chloride, the plastic that contains phthalates and is found in shower curtains, children's toys and packaging materials.

Some companies, such as Born Free LLC, a Florida-based baby bottle-maker, are promoting goods that do not contain bisphenol A. Ron Vigdor, Born Free president, said his small company is experiencing rapid sales growth.

Most consumers remain unaware of the potential dangers they are bringing into their homes, said Jane Adams, a neurotoxicologist at the University of Massachusetts.

"Most of the population would not be well-informed and necessarily know what steps to take," Adams said.

Roder, the Greendale mother who volunteered to have her house checked for endocrine disruptors, is grateful for the information she got.

Since the audit, Roder filled a garbage bin full of items that she'll no longer use - waxed paper, plastic wrap, old plastic cups, toys and containers.

She says her husband teases her for whacking bugs with shoes now, refusing to use bug spray. Instead of giving in to anxiety, Roder says her newfound awareness has brought peace of mind.

"It made me feel safe," she said.

But few people have the luxury of knowing what in their house is safe because few products contain any labeling of these compounds. Even the government scientists charged with alerting the public to the chemicals' dangers say information is sorely lacking.

"The real problem is that we don't know where all the different phthalates are coming from in our environment," said Gray, the EPA biologist whose lab has examined effects of endocrine disruptors for two decades. "I can't tell them what products to specifically avoid. The information isn't there."

From the Nov. 25, 2007 editions of the Milwaukee Journal Sentinel

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CHEMICAL FALLOUT: JOURNAL SENTINEL WATCHDOG REPORT

WARNING: The chemical bisphenol A has been known to pose severe health risks to laboratory animals. AND THE CHEMICAL IS IN YOU.

It's in baby bottles, soda cans and 93% of us. It causes breast cancer, testicular cancer, diabetes and hyperactivity in lab animals, according to 80% of studies analyzed by the Journal Sentinel. But U.S. regulators side with the chemical-makers and say it's safe.

By SUSANNE RUST, MEG KISSINGER and CARY SPIVAK
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Posted: Dec. 2, 2007

For more than a decade, the federal government and chemical-makers have assured the public that a hormone-mimicking compound found in baby bottles, aluminum cans and hundreds of other household products is safe.

But a Journal Sentinel investigation found that these promises are based on outdated, incomplete government studies and research heavily funded by the chemical industry.

In the first analysis of its kind by a newspaper, the Journal Sentinel reviewed 258 scientific studies of the chemical bisphenol A, a compound detected in the urine of 93% of Americans recently tested. An overwhelming majority of these studies show that the chemical is harmful - causing breast cancer, testicular cancer, diabetes, hyperactivity, obesity, low sperm counts, miscarriage and a host of other reproductive failures in laboratory animals.

Studies paid for by the chemical industry are much less likely to find damaging effects or disease.

U.S. regulators so far have sided with industry by minimizing concern about the compound's safety.

Last week, a panel commissioned by the National Toxicology Program released a report finding bisphenol A to be of some concern for fetuses and small children. It found that adults have almost nothing to worry about.

Its recommendations could be used by the U.S. Environmental Protection Agency and other regulators to assess federal policies on how much bisphenol A is safe and may have huge ramifications for the multibillion-dollar chemical industry.

The panel said it considered more than 700 studies by university scientists, government researchers and industry-funded chemists. It picked the work it felt was best and threw out the rest.

The Journal Sentinel found that panel members gave more weight to industry-funded studies and more leeway to industry-funded researchers.

• The panel rejected academic studies that found harm - citing inadequate methods. But the panel accepted industry-funded studies using the same methods that concluded the chemical does not pose risks.

• The panel missed dozens of studies publicly available that the Journal Sentinel found online using a medical research Internet search engine. The studies the panel considered were chosen, in part, by a consultant with links to firms that made bisphenol A.

• More and more university researchers and foreign governments are finding that bisphenol A can do serious damage in small doses. But the panel rejected studies mostly submitted by university and international government scientists that looked at the impact at these levels.

• The panel accepted a Korean study translated by the chemical industry's trade group that found bisphenol A to be safe. It also accepted two studies that were not subjected to any peer review - the gold standard of scientific credibility. Both studies were funded by General Electric Co., which made bisphenol A until it sold its plastics division earlier this year.

"This undermines the government's authority," said David Rosner, professor of history and public health at Columbia University. "It makes you think twice about accepting their conclusions."

Panel chairman Robert Chapin, a toxicologist who works for Pfizer Inc., the pharmaceutical giant, defended his group's work.

"We didn't flippin' care who does the study," said Chapin, who worked as a government scientist for 18 years before joining Pfizer.

If the studies followed good laboratory practices and were backed with strong data, they were accepted, Chapin said.

Created to act as hormone

Bisphenol A was developed in 1891 as a synthetic estrogen. It came into widespread use in the 1950s when scientists realized it could be used to make polycarbonate plastic and some epoxy resins to line food and beverage cans.

With the advent of plastic products such as dental sealants and baby bottles, the use of bisphenol A has skyrocketed. The chemical is used to make reusable water bottles, CDs, DVDs and eyeglasses. More than 6 billion pounds are produced each year in the United States.

In recent decades, increases in the number of boys born with genital deformities, girls experiencing early puberty and adults with low sperm counts, uterine cysts and infertility prompted some researchers to wonder whether the prevalence of bisphenol A could be interfering with human development and reproduction.

Scientists began looking for a link between bisphenol A and spikes in cancer, obesity and hyperactivity. Others, such as Patricia Hunt, simply stumbled onto it.

Hunt, a scientist at Case Western Reserve University, was investigating the connection between maternal age and Down syndrome in 1998 when all of her laboratory mice, including those not treated in any way, began exhibiting chromosomal abnormalities.

Her investigation revealed that bisphenol A was leaching from the animals' polycarbonate cages, and it was the chemical that had caused the problems.

Ana Soto, a researcher at Tufts University, began noticing that her lab mice treated with bisphenol A were a lot fatter than her other mice.

More alarming still was the work scientists found in their breast and prostate cancer research. They injected cancer cells in test tubes of bisphenol A and watched as the cells grew rapidly, even at doses lower than what people are normally exposed to. Reports such as these sparked fear that bisphenol A could become the new lead or asbestos.

As scientists' suspicions grew, regulators repeatedly reassured the public that the chemical was safe. The Food and Drug Administration and the EPA routinely pointed to studies by government regulators in the 1980s that found no serious effects.

In 1998, the National Toxicology Program formed the Center for the Evaluation of Risks to Human Reproduction to look at why so many people were unable to conceive or carry their babies to term. Scientists were suspicious of the environmental impact from chemicals, including hormone-mimicking chemicals such as bisphenol A.

Last year, two groups of scientists were appointed by the federal government to gauge bisphenol A's risks.

One panel was purely academic, made up of 38 international experts in bisphenol A who work for universities or governments. In an August report, they found a strong cause for concern.

Levels of bisphenol A in people were higher than the levels found to cause harm in lab animals, the panel said. The average level found was above what the EPA considered safe.

The other group, led by Chapin, included 12 scientists. The members were chosen because of their lack of detailed knowledge about bisphenol A. The idea was that the group would serve as an impartial jury, Chapin said.

It considered 742 studies conducted over the past 30 years.

The non-expert panel was less alarmed about bisphenol A's effects.

The non-expert panel's report was posted Monday on the center's Web site without a press release or fanfare. When the panel released an earlier draft, critics assailed it as arbitrary, biased and incomplete.

The sharpest response came from bisphenol A experts, many of whom had their work rejected by the non-expert panel. Even those whose work was accepted were critical of the findings.

"When panels that are sponsored by the government come out with reports and say that there is not convincing evidence yet, that gives me great concern, knowing what I do about some studies showing that there are effects," said Gail Prins, professor of physiology at the University of Illinois at Chicago and an expert in bisphenol A.

The federal government will now weigh the reports of both the expert and non-expert panels before assessing safe levels of bisphenol A.

Studies found widespread effects

Before reviewing the panel's reports, the Journal Sentinel analyzed 258 studies spanning two decades. All studies involved live animals with spines - those species scientists consider most relevant to people. The studies were found on

PubMed, an online search engine used by researchers.

Four out of five studies found that bisphenol A caused problems in the lab animals tested, ranging from allergies to reproductive deformities. The vast majority of these studies were funded by government agencies and universities.

One federally funded study found that rats exposed to bisphenol A before birth were at increased risk of developing precancerous prostate lesions. Another study, funded by the U.S. and Argentine governments, found that the chemical increased the likelihood of rats developing mammary tumors.

Just 12% of the studies found that bisphenol A had no ill effects. Most of those studies were paid for or partially written by scientists hired by the chemical industry.

A study funded by the Society of the Plastic Industry found that bisphenol A did not pose harm to developing rats. Another study discounted any reproductive effects on exposed rats. The authors included scientists affiliated with Shell Chemicals, Dow Chemical Co. and General Electric - all then makers of bisphenol A.

Two studies actually determined that bisphenol A may be beneficial. One funded by drug-maker Eli Lilly & Co. said it could lower cholesterol in rats. The other study said the chemical might prevent or cure breast cancer in rats.

Industry scientists dispute any claims that bisphenol A is harmful to humans.

"Our view is consistent with what has been concluded by government and scientific bodies around the world, which is that bisphenol A is not a risk to human health based on the weight of scientific evidence," said Steven G. Hentges, executive director of the American Chemistry Council's Polycarbonate/BPA Global Group. Hentges called the newspaper's review superficial.

Norman Fost, founder and director of the medical ethics program at the University of Wisconsin-Madison, said industry and academic studies come to radically different conclusions all the time. Fost would not comment directly on the panel's work because he hadn't studied it. But he said the universe of scientific research is replete with studies conducted by organizations with a vested interest.

"It's up to us to be skeptical, cautious and critical when we consider how much of their work to believe," said Fost, who is chairman of an FDA committee looking at the ethics of pediatric studies.

Human safety levels

Bisphenol A is just about everywhere. But trying to get a handle on how much of the chemical a person can tolerate is not easy.

The government established a safety level for bisphenol A about 20 years ago - well before most scientific studies on the chemical had been conducted. The government considers a safe daily level of bisphenol A to be 50 micrograms per kilogram of body weight. For a 200-pound person, that would be the equivalent of no more than one drop of the chemical every five days.

The American Chemistry Council says an average adult would have to ingest more than 500 pounds of canned food and beverages every day for an entire lifetime to be at risk. The chemical industry based those conclusions on its own research.

Others say there is no way to know how much bisphenol A one is exposed to when microwaving dinner in a plastic container, eating tuna from a can or drinking from a reusable plastic water bottle.

"Even if you go out of your way to avoid products, you don't know all of your exposures," said Soto, the bisphenol A expert from Tufts. "At the end of the day, you may have cut your exposure by 5 percent or by 95 percent. We just don't know."

Because bisphenol A is so ever-present in the environment, there are many ways to be exposed to it. But the biggest risk comes from those products that people put in their mouths or that come directly into contact with food, scientists say.

A number of studies looked at how bisphenol A affects lab animals at low doses. Bisphenol A experts say that the chemical works like a hormone and, therefore, needs to be tested at low doses where much damage can be done.

"This is basic endocrinology," said Frederick vom Saal, a biologist at the University of Missouri who has been studying bisphenol A for more than a decade. "You learn this in any introductory class. Hormones work on an extremely sensitive system."

For instance, it only takes 40 parts per billion of the hormone MIS to produce male sexual organs in the human embryo. That's about one drop in 15 bathtubs of water.

Two groups of scientists - from the National Academy of Sciences and the National Toxicology Program - have called for the U.S. government to radically overhaul the way it tests chemicals to include these low doses. But the government has yet to do so. Instead, it continues to cite the government studies from the early 1980s that focused only on high doses.

Of the 258 studies reviewed by the Journal Sentinel, 168 studies looked at low-dose effects of bisphenol A.

The vast majority - 132 studies- found health problems at low doses, including hyperactivity, diabetes and genital deformities. All but one of those studies were conducted by non-industry scientists. Nearly three-fourthsof the studies that found the chemical had no harmful effects were funded by industry.

But Chapin's panel did not accept any studies that found an effect at low doses in its review of 742 studies.

Once the panel weeded out studies it believed had been done poorly, no studies remained that showed effects from low doses, Chapin said.

"There's a lot of bad science out there," he said.

Most of the low-dose studies the Journal Sentinel reviewed - including some the panel rejected - were published in reputable scientific journals.

Prins, the bisphenol A expert from the University of Illinois at Chicago, said she was a late convert to the idea that the chemical causes harm at low doses. She changed her mind after reading repeated studies.

Then she saw it in her lab.

"We gave very small doses to male rats and saw cancerous lesions form on their prostates," Prins said.

For the panel to dismiss low-dose effects is a fatal flaw, she said.

Chapin conceded that the panel did not give equal weight to studies that considered low-dose effects, the levels that most people are exposed to every day.

"I'll admit it. We may be off in like totally uncharted territory," Chapin said.

The chemical industry defended the panel's choice of studies, noting that their scientists have been unable to replicate the work of some university scientists.

"Replication is a hallmark of science, and studies that cannot be replicated cannot be accepted as valid," said Hentges of the chemistry council.

Panel's work studied

The Journal Sentinel reviewed the work that the panel did, comparing each of its two drafts and the final report, together totaling more than 1,000 pages.

Two of the panel's four chapters considered the same kind of studies the newspaper reviewed - looking at the effects of bisphenol A on live animals. In one of those chapters, focusing on reproductive toxicology, 20 studies by either government or academia were tossed. No study that disclosed it had been funded by industry was rejected.

Chapin said they gave greater weight to studies that used more animals. Critics say only the chemical-makers can afford to conduct studies with more animals.

The panel failed to apply consistent standards, the newspaper's review found.

Not all studies recorded the kind of feed, caging, bedding or specific type of animal used. Those factors can influence the studies' results.

Chemical industry researchers used the same methodology in studies the panel accepted that caused other studies to be rejected. They included studies that used a single high dose and injected rats with bisphenol A rather than having the chemical administered orally. Chapin's panel rejected some studies, including those conducted by Soto, because they used an oil called DMSO to administer bisphenol A to rats.

"That just helps compounds waltz into cells," Chapin said.

But Chapin's panel accepted another study that used DMSO, never citing that oil as a limitation or concern.

The panel also accepted a study by Shell Chemical, Dow Chemical and General Electric that found no effects from bisphenol A. The same study also found no effects when rats were exposed to the powerful chemical diethylstilbestrol, or DES - a compound known to cause reproductive harm.

The rats' resistance to DES should have been an immediate red flag, critics said. But the panel accepted the research.

Consulting firm fired

Chapin's group has been dogged by controversy from the beginning. Last year, conflict-of-interest concerns were raised regarding the panel's use of Sciences International. The Virginia-based consulting firm had been hired to choose and summarize research for panel members. However, it had not been revealed that Sciences International had clients that included bisphenol A producers.

The company was fired in April, and the National Institutes of Health audited the firm's report. It found no conflict, and the company is credited in the final report.

Chapin dismissed criticisms against the panel.

"I'm tired of having my credibility as a scientist questioned when the panel bent over backwards to apply standards of good scientific conduct . . . evenly across the board," Chapin said. "My accusers have a great deal more bias than I do.

"They are not unbiased," Chapin added, "even though they keep holding themselves up as the white hats, the pure, the only holders of the cup of scientific chastity."

The newspaper found dozens of studies of bisphenol A that were not brought to the panel's attention.

Among them was a 2005 study that determined the chemical disrupted brain development in rats at very low levels. The panel also missed a study last year by Yale University researchers that found the chemical altered reproductive tract development in female mice exposed in the womb. Again, the researchers found these effects at low levels - below what the EPA considers safe.

"I'm surprised because my understanding was after all the hoo-ha was raised about Sciences International, the NTP went out and did its own search," Chapin said. "That's weird."

In one study accepted by Chapin's panel, the work was translated into English by the American Plastics Council, a division of the American Chemistry Council. The Korean study found that the sperm density and the reproductive systems of male rats were not harmed by bisphenol A.

Rosner, the public health professor, said that practice "immediately raises eyebrows."

"You have to have a neutral party doing the translations," he said. "It's the only way to really trust the accuracy."

Michael Shelby, director of the government agency that selected the panel to evaluate bisphenol A, acknowledged that the translation could be called into question. However, he denied any conflict.

Chapin said panel members agreed that they wanted to see any data they could, regardless of how they got it.

"I hear what you're saying about the perception," Chapin said. "Too bad."

Two studies, both funded by industry, were not peer reviewed, the newspaper found. Peer review is considered the foundation of scientific credibility. Most scientific journals will not publish a study unless it is peer reviewed.

The studies found no effects from bisphenol A, and were funded by General Electric in 1976 and 1978. They were accepted despite concerns similar to those that led the panel to disqualify academic and government studies. They included a small sample size of animals, the use of high doses and questions about the statistical methodology.

The panel also accepted at least a dozen studies that had not been published in any scientific journal - another check and balance in the scientific community to maintain high standards.

Shelby said the panel considered studies that were not peer reviewed if they included sufficient details.

Even scientists on the panel who agreed with the findings say they are uneasy about broad claims that bisphenol A is safe.

Jane Adams, a professor at the University of Massachusetts, doesn't allow her teenage son to get dental sealants because of her worries about bisphenol A.

"I am concerned about this chemical," she said. "Much more research needs to be done."

Simon Hayward, another panelist, agrees.

"Where there's smoke, there's fire," said Hayward, professor of prostate biology at Vanderbilt University Medical Center. "There is definitely enough smoke to be worried."

Rosner, the public health historian, says bisphenol A's potential for danger is too great to allow its widespread use without being certain of its safety. Consider what happened with lead and tobacco, he said.

"The government needs to work with caution," he said, noting that we have lived well for thousands of years without this chemical. "Until we know that it is safe, it is more prudent to avoid it."

From the Dec. 2, 2007 editions of the Milwaukee Journal Sentinel
Have an opinion on this story? Write a letter to the editor.

Exhibit G

TRANSCRIPT

May 23, 2008

BILL MOYERS: If it weren't for the work of a muckraking journalist more than a century ago, the Federal Food and Drug Administration, the FDA, might never have existed. In 1906, Upton Sinclair published The Jungle, a fierce graphic account of the meatpacking industry's filth, corruption and exploitation. His vivid, stomach-churning depiction fueled a demand for more stringent inspections and the creation of the FDA to keep food, medicine and other products fit for human consumption.

Last week, FDA officials and other government witnesses were called to a Senate Committee hearing on the safety of Bisphenol A, or BPA that's a chemical used in a variety of plastic products from baby sippy cups to eyeglasses. When Senator Schumer and five of his colleagues introduced legislation calling for a ban on BPA in all kids' products, they used information uncovered by the investigative reporting of the Milwaukee Journal Sentinel. How the paper's reporters got that story is the subject of this report from our colleagues at Exposé, narrated by Sylvia Chase.

NARRATOR: Plastic. It's in almost everything. Food is stored in it. People drink from it...work with it...play with it. Less well known is that there is a chemical contained in many plastics that is also found in 93 % of us.

It's called "Bisphenol A."

FREDERICK VOM SAAL: Bisphenol A is actually the chemical used to make polycarbonate plastic. It's the hard, clear plastic used in baby bottles, and it also is the lining of all metal cans made in the United States - beer cans, soda cans, food cans. And this chemical leaches out of all of these products into any kind of food or beverages that come in contact with it.

NARRATOR: Bisphenol A, BPA, is what is known as an endocrine disruptor. Studies have shown that in lab animals, it causes breast and testicular cancer, diabetes and hyperactivity. Its effects on humans are not entirely known. The manufacturers of BPA, and their lobbyists, say it is safe. U.S. regulators agree. One team of investigative journalists decided to ask...why? Susanne Rust is a science reporter with the MILWAUKEE JOURNAL SENTINEL.

SUZANNE RUST: When I was a graduate student, I read this article in the New Yorker. The theory was that there were chemicals in the environment that were somehow messing with the reproductive system. And then I got into journalism, and suddenly these science stories kept coming across my desk. The managing editor of my paper was really excited about one of the stories I had written. It was on this chemical, Bisphenol A, and he was like, "You interested in this?" I said, "Yeah."

GEORGE STANLEY: I went to Susanne and said, "We know breast, prostate and other forms of these cancers related to the endocrine system are on the rise in humans. We know this stuff causes it in lab animals. We've got to look into this."

TALKBACK: THE BLOG

Our posts and your comments

OURPOSTS

May 23, 2008
Ask the Reporters: Exposé on Bill Moyers Journal...

YOURCOMMENTS

"In response to SN- I have idiopathic severe primary RLS- since childhood. I was finally diagnos..." - *David Robinson*

MARK KATCHES: Well, I think the central question that we came up with from the get go was, "Why isn't anything being done to address the issue?" So we set our sites on the regulatory efforts, what the EPA had been doing. It turns out, not much.

NARRATOR: Endocrine disruptors were first identified as the cause of wildlife abnormalities in the early 90s. The Environmental Protection Agency and the Food and Drug Administration, though, repeatedly reassured the public that BPA, at least, was safe.

The agencies cited studies done in the 1980s. But prompted by an outcry from advocacy groups, President Bill Clinton signed the Food Quality Protection Act in 1996. That same year, the Safe Drinking Water Act was amended.

The combined legislation promised a chemical screening program of endocrine disruptors to be overseen by the EPA. The goal was to determine whether or not they were dangerous to human beings.

1998 - the EPA, headed by Carol Browner, sets a deadline to fast track the testing of 15,000 chemicals suspected as endocrine disruptors....

1999 - the EPA misses the deadline. The Natural Resources Defense Council sues the agency to enforce screening. 2001 - a new administration takes office; Christine Todd Whitman becomes head of the EPA.

2003 - two more suits are brought against the EPA, one by a coalition of environmentalists and advocacy groups...the other by the attorneys general of four states. The suits attempt to force the agency into compliance with the food quality protection act....

2007 - 11 years after the laws were passed...the EPA had yet to screen its first chemical.

Mark Katches assigned two members of what the paper calls its "watchdog team" to join Susanne Rust in exploring why: Cary Spivak...and Meg Kissinger.

MEG KISSINGER: You can go as, you know, walk into any grocery store or go to any makeup counter, and you know, you'll find plenty of products that contain chemicals that are suspected, and that's the big word, you know, they're suspected of health concerns. And the mainstream media just hasn't paid that much attention to it.

NARRATOR: The Journal Sentinel began to give some attention to the Environmental Protection Agency in June of 2007.

CARY SPIVAK: At first, they were very cooperative, but when we said, "Look, that Congress passed a law saying you're supposed to be screening these chemicals, and you keep pointing out that you're working on it, but now it's 2000 - at the time 2007 - you have yet to screen a chemical." And they realized that we were really pushing them and demanding answers. They got much more difficult to deal with.

NARRATOR: Stephen Johnson is the head of the EPA. The paper would report he declined repeated requests for an interview.

MARK KATCHES: And we're the Milwaukee Journal Sentinel. We're not The Washington Post or The New York Times. So when you start calling officials from the EPA, you're not going to get the same kind of attention that, that those newspapers would get. So we had to be really persistent.

CARY SPIVAK: There was a period of time where they just said, "We've answered all your questions." We said, "We don't care if you think you answered all our questions." We just kept going. And even on basic things they were very difficult to

deal with.

NARRATOR: The reporters eventually learned that the EPA, though it hadn't tested a single one of the 15,000 chemicals promised, had already spent some $80 million on the endocrine disruptor program.

GEORGE STANLEY: And here's tens of millions of dollars, our tax dollars being spent and not a single chemical has ever been tested, to this day. So, the more they dug, the more they found.

NARRATOR: He team also learned that only in 2008 did the agency plan to screen its first chemicals- just 73 of them, and not including BPA...and they wouldn't even be finished with that until 2010.

CARY SPIVAK: That took literally weeks to get answers from them to give us the date of 2010.

NARRATOR: Elaine Francis, the national program director of the agency's endocrine disruptors research program, told the paper:

"Clearly, we would have liked to have been a lot further along. But science tends to move at its own pace." **NARRATOR:** But the pace of science was far from the only issue. The reporters wanted to know: just what was the science saying? The journalists found two camps, each with its own view of the science of Bisphenol A. One includes Dr. Frederick Vom Saal.

FREDERICK VOM SAAL: In our test system with human breast cancer cells, what we found with Bisphenol A was very different than what happens with a natural hormone produced in your ovaries.

NARRATOR: Vom Saal is a biologist at the University of Missouri. He has studied Bisphenol A for more than a decade. In 1997, a team of researchers led by Vom Saal published a peer-reviewed study showing that when BPA was introduced to human breast cancer cells, it penetrated the cells and made them grow rapidly.

FREDERICK VOM SAAL: And as a result of that, we got interested that maybe this chemical was a lot more potent than anybody had previously thought. And so we did a study where we administered it to mice, and found that at a dose 25,000 times below what anybody had ever tested, we caused damage to the entire developing male reproductive system.

NARRATOR: Chemical companies who make or have made Bisphenol A say that people have little or nothing to fear from what are known as endocrine disruptors.

MEG KISSINGER: The chemical companies' basic answer was, "There's no known direct effect that these chemicals are harming anybody."

NARRATOR: The paper heard the same from the industry's powerful trade and lobbying association, the American Chemistry Council. The ACC's Marty Durbin said: "Science supports our side...."

An industry consultant and former EPA regulator, James Lamb, agreed, saying, "I'm very comfortable with my kids and grandkids using these products...because i believe the industry has done the studies that need to be done and that they're interpreting them properly."

In defense of the safety of Bisphenol A, the companies and the ACC cited studies they funded themselves, some paid for by the ACC, which has an annual 75 million dollar budget.

SUZANNE RUST: They say the reason they get these, these results is that their

studies are better than any of the academic studies, any of the government studies. They can use more animals; they have better controls in their laboratory.

NARRATOR: But one EPA biologist, L. Earl Gray, Jr., charged the industry with flooding the EPA with studies. David Rosner, professor of history and public health at Columbia University, explained why, telling the paper chemical makers have "... learned that if you play on the uncertainty of danger, you're going to be able to stop regulatory action..."

CARY SPIVAK: What you have, is you have these studies will come out, and they have to weigh that against the academic studies or other studies that are questioning it. And if nothing else, the more you give the EPA additional studies, the more time it's eating up. The more time it's eating up, the more you're selling your product. You've won.

NARRATOR: The ACC's Marty Durbin denied that industry tries to stall the EPA's work. The paper posted this interview with him on its website.

DURBIN AUDIO: "If it was our interest to delay things around here, we'd just sit on our hands and see whether or not EPA gets any funding. But we actually, year after year, go up to the Congress using our resources and lobby to have essential funding to the EPA for these particular research programs. So, again I think our record, our record speaks for itself. We've been fully supportive of moving this process along."

NARRATOR: Again and again, the reporters heard two different stories. One example: they found a statement on an ACC-sponsored website. It said that a person would have to ingest over 500 pounds of canned food every day to be at risk from BPA found in the containers.

Other scientists told the reporters that even at very low doses, BPA and similar chemicals can affect lab animals...the concern is that they might harm human beings, too.

SUZANNE RUST: It surprised me too how much rancor there was about this chemical. I mean, you would talk to some scientists, and you know, they would tell you that the sky was falling. I mean, we talked to others, and they would tell you that it was fine, and then in the same sort of breath they would cut the first scientist down personally. I mean, it was just kind of amazing. I felt like I'd walked into sort of a geeky chemistry war zone.

NARRATOR: After three months of reporting, Rust, Kissinger and Spivak pulled together the information they had culled about the debate over endocrine disruptors.

MEG KISSINGER: And I remember we had a couple of stories sketched out, and we were pretty happy with them, and it was really basically saying, "There's all these chemicals out there, the government's not testing them as they promised they would. A lot of other countries are much more diligent about this." And then here's kind of a lot of the infighting. So we turned these stories in, and we're all excited, and kind of like, "Oh, oh okay, well, that's a wrap. " And not at all. We were called into Mark Katches' office, and basically got our fannies handed to us on a platter. And he just said, you know, "You're not there, yet." So, we were crushed.

NARRATOR: The story was at an impasse. The editors wanted more work. More investigation. More examination of the science.

MARK KATCHES: We realized that the story would have a lot more authority if we went back and looked at all of the studies that had been done, and really tried to conclusively show, is this a problem, or is it not?

NARRATOR: Before she had become a journalist, Susanne Rust had been a graduate student in Biological Anthropology. Now the paper would call on her experience with scientific methodology.

SUZANNE RUST: I'm not intimidated by scientific studies, right, I'm not afraid to read a methods section. I'm not afraid to read results section. I had enough through background in endocrinology, where I was fairly familiar with the terms they were using.

BECKY LANG: So Mark was like, "Why we don't just do our own analysis?" And so he turned to Susanne and said, "Do you think you could do this analysis?" Yeah, she thought she could.

NARRATOR: To begin her research, Rust headed back to school...to the UW Madison library where she had done her graduate work.

SUZANNE RUST: I searched for Bisphenol A, looking for those criteria which I had initially set out for myself, which were live laboratory animals with spines. Where were the authors, what institutes did they work for, who funded the study, what the author's conclusions about the chemical were, how many animals they used to come up with these conclusions...

NARRATOR: Rust also turned to another public source of medical and scientific studies of Bisphenol A, done by both industry and academic scientists.

SUZANNE RUST: I went to PubMed, which is a database online that sort of puts together all medical and scientific studies, and I put together a huge database with all of this information.

NARRATOR: In all, Rust evaluated 258 studies done over two decades involving lab animals with spines, the type scientists consider most relevant to human beings.

SUZANNE RUST: Right away, you could see that 80% of these studies all found that this chemical caused harm.

NARRATOR: More than half the studies, 168 of them, evaluated Bisphenol A at low doses. The vast majority of those - 132 of the 168 - showed harm to lab animals. And, Rust would report, "nearly three-fourths of the studies that found the chemical had no harmful effects were funded by industry." Rust's overall conclusion: an overwhelming majority of the studies found BPA to be harmful in lab animals - causing breast and testicular cancer, diabetes, hyperactivity, obesity, low sperm counts, miscarriage and other reproductive failures. Studies paid for by the chemical industry were much less likely to find damaging effects or disease.

MARK KATCHES: That's where this story took on a whole different dynamic. Because you were able to show, conclusively, through that analysis of all those studies, that hundreds of researchers across the world had found problems with Bisphenol A. And yet, nobody had done anything, and only a few studies had found that it was safe. And most of those studies were funded by the chemical industry themselves. And, and that's when you knew you had something really, really special to tell to readers.

NARRATOR: All of the studies Rust had evaluated were in the public domain, as available to government regulators as they were to a reporter in Milwaukee. As the reporters were working on their story, they knew the government was continuing to look into Bisphenol A. They would report on one panel funded in part by the National Institute of Environmental Health Sciences. It consisted of experts who directly studied the chemical. That panel found in 2007 - quote - "great cause for concern" about BPA.

Meanwhile, the National Toxicology Program, the NTP, was in the process of coming up with its own brief on Bisphenol A.

Part of the Department of Health and Human Services, the NTP evaluates chemicals and other agents of public health concern. In 2007, the NTP convened a panel composed of scientists who didn't directly study BPA, but would evaluate the work of

those who did.

Among the panel's conclusions: While for pregnant women, fetuses and children there is some concern about neural and behavioral effects...there is minimal concern for... ...prostate effects... ...potential accelerated puberty... ...there is negligible concern for birth defects and malformation.

For adults, the concern was essentially negligible. In light of her own findings, Susanne Rust wondered how the panel had arrived at those conclusions.

SUZANNE RUST: We pulled out every single study looked at in their review of Bisphenol A studies. And so we just wrote down what the study was, who funded it, was it government, industry? And then, more specific, what government agency funded it, what industry funded it, what kind of animal did they look at, what was the strain, what were the doses used?

NARRATOR: Among the paper's findings: Some of the studies the NTP panel considered were chosen by a consultant with links to firms that made Bisphenol A. The panel rejected academic studies that found BPA harmful, citing inadequate methods, but accepted industry-funded studies using the same methods...to conclude the chemical does not pose risks. It also accepted two studies finding no harm funded by former BPA-maker General Electric. They were done some 30 years ago. Neither was peer reviewed.

MEG KISSINGER: I try not to be too cynical, but I don't trust that. I would rather have an independent entity testing the stuff to know, versus the guy that's making it.

NARRATOR: And the panel didn't accept any studies that found BPA harmful at low doses. Why? The paper reported the panel's chairman, Robert Chapin, said that once the panel weeded out studies it believed had been done poorly, no studies remained that showed effects from low doses.

Chapin is a toxicologist who has worked in both government and industry. He defended the panel's work, saying that it had accepted studies that followed good lab practices and were backed with strong data, regardless of where they originated. He told the paper, "We didn't flippin' care who does the study."

In November 2007, the reporters rolled out a two-part series entitled "Chemical Fallout."

Among its conclusions: the government's contention that BPA is safe is based on outdated, incomplete government studies and research heavily funded by the chemical industry.

CARY SPIVAK: And we said, "Why this is important to you. Why you should care about what's in the containers holding your food or other products. And that this is all over the place and that there are legitimate scientific questions over the safety."

GEORGE STANLEY: We saw immediate reaction in Milwaukee in the market place. As soon as mothers read this, they stopped buying baby bottles that had this plastic. And they had to go order a bunch of baby bottles made of glass, and BPA-free plastic.

MARK KATCHES: We have not gotten a single demand for retraction, no clarification request from the chemical industry. They've had nothing that they could come back to us on.

NARRATOR: That doesn't mean the industry has stopped defending Bisphenol A. In answer to a question in an online chat the paper sponsored, ACC spokesperson Stephen Hentges wrote:

"It is not correct that only industry studies support the safety of products made from Bisphenol A...government and scientific bodies with no stake in the matter have impartially reviewed all of the scientific evidence to reach their conclusions. The recent NTP panel evaluation is a good example."

CARY SPIVAK: A lot of these plastic products, people like. It makes life more convenient. The battles are going to become more intense as time goes on. You're having more studies come out raising questions about it.

GEORGE STANLEY: We still don't have the answers to a lot of the questions. And we'll be continuing the investigation.

MEG KISSINGER: This is Meg Kissinger calling with The Milwaukee Journal Sentinel...

BILL MOYERS: On April 15th, the National Toxicology Program, the NTP, going beyond its own panel's preliminary conclusions, issued a brief, it stated, in part: "... the possibility that Bisphenol A may alter human development cannot be dismissed."

THE MILWAUKEE JOURNAL SENTINEL reported that this was the first time a federal agency has acknowledged that BPA is potentially dangerous to humans.

Meanwhile, Canada's government has announced its intention to ban the sale of plastic baby bottles containing BPA and last Thursday, the California State Senate voted to forbid the use of BPA in childcare products.